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Final Report

NEW TREATMENT COMBINATIONS FOR CONTROL OF BRUSH AND VEGETATION MANAGEMENT ALONG INDIANA ROADSIDES

James D. Morré

PURDUE UNIVERSITY

Final Report

NEW TREATMENT COMBINATIONS FOR CONTROL OF BRUSH AND VEGETATION MANAGEMENT ALONG INDIANA ROADSIDES

FHWA/IN/JHRP-95/3

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Purdue University
West Lafayette, IN 47907
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16. Abstract

This report represents results from a project for research to develop and implement new treatment mixtures for control of problem brush, trees and other woody species and herbicide-resistant weeds along Indiana roadsides. An environmentally safe mixture of trichlopyr (Garlon Herbicide) and ammonium nitrate for one application control of deciduous roadside vegetation was developed. Also discovered and developed was a new, environmentally safe and effective TR series of additives that enhance the action of the auxin herbicides (trichlopyr, 2,4-D, picloram) for inclusion in both the brush control mixture and for possible use for the control of milkweed, canada thistle, bindweed, ground cherry and other perennial, herbicide-resistant roadside weeds.

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roadside maintenance, vegetation, brush, trees, noxious weeds, weeds, environmental safety, herbicides, growth retardant mixtures

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1) Identification

a) Title - NEW TREATMENT COMBINATIONS FOR CONTROL OF BRUSH AND FOR GENERAL VEGETATION MANAGEMENT ALONG INDIANA ROADSIDES

b) Organization - Department of Medicinal Chemistry
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West Lafayette, IN 47907

c) Principal Investigator - D. James Morré, Dow Distinguished Professor

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2) Introduction

Review of literature.-- Unwanted roadside vegetation has important implications in roadside management in terms of safety and long term costs (Ross, 1981; Johnson, 1986; Gould, 1987; Talbot, 1987; Burkhardt and Morré, 1986). Excessive vegetation growth can obscure sight lines or even cover important road signs and warnings. Such areas are unsightly, harbor weeds noxious to agriculture and are proven more costly to maintain if only mechanical methods are used than when mechanical methods are supplemented by chemicals. Often cost reductions of 20 to 50% can be realized with full implementation (Pourciau, 1980; Morré, 1981; Richard, 1986; Dixon and Dickens, 1986; Raczon, 1988; Jett, 1988). However, there are a number of instances where mechanical methods are not feasible an it is here where chemicals may have their most important and perhaps least well evaluated roles (Evans, 1986; Miller and Middlebrooks, 1987; Zarichnak et al., 1986).

There is a surprisingly small amount of published research in recent years pertaining to roadside vegetation management and especially on the subject of brush control. A compendex Plus Search from 197--1989 provided only 15 citations, of which 10 were of some relevance and one was to our own published contribution to the subject. DIALOG searches from the TRIS and NTS data bases yielded 28 entries, 3 to our own work, and 12 entries, 2 to our own work, respectively. More than 10 years have passed since the last comprehensive evaluation of brush control herbicides and application methods for roadside use in Indiana was completed (Morré, 1980).

Much of the brush control literature pertains to forest management and release from competition or control of undesirable species in forest plantings (Byrnes and Holt, 1988; Elmore, 1987; Minoque and Zutter, 1986; Minoque et al., 1983; McLemore, 1983; Hurst and Blake, 1987; McCollum, 1987; Gonzalez, 1983; Fitzgerald and Forston, 1981; Seifert and Holt, 1985). While considerable important information has been obtained from these sources, the objectives are sufficiently different to preclude direct transfer of most recommended practices for forestry management nationwide to roadside situations in Indiana.

A survey of brush control literature in non-cropping situations revealed published work dealing with effects of different herbicides on single species such as multiflora rose (Cresswell, 1984; Underwood and Sperow, 1986; Underwood et al., 1983), Japanese honeysuckle (Regehn and Frey, 1988), autumn olive (Kuhns, 1986), rough-leaf dogwood (Fick and Janicke, 1984), honey mesquite (Meyer and Bovey, 1986) or turkey oak (Cantrell et al., 1984), as examples. These were of little value to roadside applications in Indiana. The bulk of the remainder dealt with just two closely related herbicides, Tordon (picloram) and Garlon (triclopyr) (Larelle, 1986; Reid, 1987; Jackson, 1987a, b; Kline and Hern, 1985; Hall and Hendler, 1985; Melichar and Geyer, 1983; Melichar et al., 1985; Melichar et al., 1986; Hall, 1984; Cresswell et al., 1984; Schutzman et al., 1986; Brock, 1988; Kidd and Kline, 1986). Other reports concerned telbuthiuron (Meyer and Bovey, 1988) hexaxinone (Atkins and Link, 1988; Minoque et al., 1983; Michael, 1980, 1981; McLemore, 1983; Fitzgerald and Fortson, 1981; Gonzalez, 1983; Hurst and Blake, 1987) and glyphosphate and sulfometuron methyl (Voth et al., 1986). No report dealt with mixtures of the type proposed for development under this project.

Different application methods (Waddington and Bittman, 1987; Hall, 1984; Michael, 1980, 1981; Cresswell, 1984; Dixon and Dickens, 1986; Kidd and Kline, 1986), including applications to cut stumps (Brown, 1988; Johansson, 1988; Seifert et al., 1983) and basal bark (Yeiser and McLemore, 1986) applications showed considerable utility and will be considered as part of the work program proposed.

Different surfactants can also affect the performance of brush herbicides (Tabbush et al., 1986; Balneaves, 1985). There has been no recent work using surfactants on midwestern brush species or on brush in Indiana, however.

There is general agreement from all published accounts that removal of trees and brush as well as the subsequent control of sprouts is a major economic factor in the total cost of vegetation management along roadsides. When left uncontrolled, woody vegetation quickly reduces sight distance, increases the possibility of collision with wildlife, pedestrians or other objects, chokes out grasses and other ground cover species that contribute to erosion control, stops up drainage facilities, may produce shady spots that contribute to build-up of ice and snow in winter, and no matter what the hazard, will necessitate an expensive reclearing project.

Control of woody vegetation may be carried out by mechanical means or through the use of chemicals providing that the chemicals are effective and environmentally safe. Most efficient control by either method is achieved if the woody plants are treated or removed when they are small, and before they destroy or weaken turf and ground cover by shading and competition for available water and nutrients. An aggressive research program in this area will aid the Indiana Department of Highways by developing and identifying more effective, safer, environmentally sound, and especially, more economical means of controlling brush, trees and other unwanted roadside vegetation.

Mechanical methods.--Mechanical control of brush and trees uses heavy mowers, mechanized brush "axes," or manual labor. In some areas this removal of brush must be done every year. In other areas, a two- or three-year mowing cycle could be practiced. However, no matter what, if mechanical control method is used, the process must be repeated on a regular cycle with more and more sprouts being produced after each mowing or cutting.

Mechanical axes are useful for side trimming operations or in areas that cannot be reached by mowers. While effective, they are quite expensive. Manual clearing is also effective on small areas but this method is both expensive and hazardous. With larger trees, mechanical means may be the only effective removal method.

Chemical methods.--With chemicals, the primary advantage is that they destroy the roots of woody plants as well as the stems, so that no further treatment is necessary.

Woody plants are easier to kill when small, which reduces the total expenditure. Woody plants killed at an early age are not large enough to be unsightly when sprayed or cut. Early treatment removes the woody plants and allows desired vegetative cover to become established sooner. The ten most used chemicals for roadside use are Roundup, 2,4-D, Oust, Benval, Tordon, Krenite, MSMA, Telar, Embark and Krovar (Transportation Research Board, 1988).

The principal methods of herbicide application for brush control have been reviewed previously (Morré, 1980). They include:

1) Foliage - Chemical mixtures are applied in a water solution together with a suitable wetting agent during the growing season to the leaves of woody plants. Volumes of as low as 25 gallons per acre, or up to 250 gallons per acre may be applied. For low volume applications, low pressures with a fixed nozzle, produced uniformly large droplets which could be delivered accurately and evenly to a given area (Kidd and Kline, 1986). A handgun or other sprayers equipped with a large orifice were best for higher volumes. However, the high pressures sometimes necessary to deliver the large volumes may result in small, fine droplets that drift out of the target area and caused damage on adjacent vegetation. Most commonly used woody plant herbicides work well with the foliage method. These include: 2,4-D, Tordon, 2,4-DP, Silvex, Banvel and others. Some types of wetting agent will enhance the effectiveness of these herbicides (Balneaves, 1985; Tabbush et al., 1986).

2) Stem Foliage - In the stem-foliage method of application, the stems, leaves, and root collars are wet with herbicides in water. Application rates require 100 gallons or more per acre. A handgun supplied by a high volume pump is the usual type of equipment. Stem foliage treatment is often used where brush is two or three years old or for chemical trimming. The resulting "brownout" may be unsightly. Krenite has been reported not to have this "brownout," but is effective only on a few roadside species (Morré, 1980).

3) Basal Stem - Basal stem treatments are applied to individual stems using concentrated mixtures of herbicides at application rates of 10 to 100 gallons of total spray mixture per acre (Yeiser and McLemore, 1986; Brock, 1988). The actual amount applied will depend on the strength of the mixture and on the number of stems per acre. Basal stem treatments may be applied at any season of the year but are easier when leaves are not present and the stems are more easily reached (Underwood et al., 1983; Hall and Hendler, 1985). Because the method is very labor intensive, it is rarely used to treat large area right-of-way. However, it requires inexpensive equipment and very small supply tanks and has utility for roadside purposes. A carrier, such as diesel oils or mixtures of

oils and other carriers, are used to help penetrate the bark (Melichar and Geyer, 1983; Melichar et al., 1985, 1986). Winter applications utilize carriers that do not freeze. Herbicides amenable to woody plant control by basal stem treatment include Garlon, Banvel, Silvex, Arsenal, 2,4-DP, and mixtures of these together with 2,4-D.

Basal stem applications using ultra low volumes, often will result in good control of woody plants. Mixtures containing 10 - 30% pure concentrate are applied in amounts as low as 10 - 15 cc per stem. A 2 - 3 gallon knapsack sprayer is the only equipment needed as a relatively large area is covered by a very low volume. The method finds application in areas that are inaccessible to mechanical sprayers. When applications are made during the dormant season, little brownout results. It can be used in ornamental plants and in areas such as rest areas that may be environmentally sensitive.

Dormant applications of water-based mixtures containing 2 - 3% wetting agents to penetrate the bark can be used on woody stems in early fall and late spring when freezing of the spray material is not a problem. High volumes of 50 - 100 gallons per acre are necessary to assure adequate coverage of the entire stem. Glyphosphate, fosamine, triclopyr, and imazapyr are among the materials which have performed well with this method of application.

4) Aerial applications of woody plant herbicides is effective for brush control (Hall, 1984), but is not used extensively for highway applications. It is designed for use as a foliar spray employing the Microfoil (TM) Boom for drift control. The primary use has been along power lines and for forestry uses.

5) Cut stump treatment involves application of herbicide solutions to stumps of trees after they have been cut. This method is never 100% and retreatment is soon required. Large stumps are easier to kill than smaller ones. Small stumps are easily missed as well.

In one of the more complete studies to date, control of regrowth from cut stumps of 4 species of elm (*Ulmus* sp.), sweetgum (*Liquidambar styraciflua*), sugar maple (*Acer saccarum*) and sassafras (*Sassafras albidum*) with 12 herbicide treatments applied in spring or autumn was investigated by Seifert et al. (1988). Triclopyr, picloram, glyphosphate or dicamba alone or with oil, surfactant or 2,4-D gave 57-91% regrowth control. Average control of *Ulmus* sp., *L. styraciflua*, *A. saccharum* and *S. albidum* over all herbicide treatments was 98, 65, 61 and 45% respectively. Control of *S. albidum* regrowth with triclopyr, picloram, dicamba + oil and CN0309 was 93, 71, 65 and 64% respectively. Control of regrowth with herbicides giving 70% control with application immediately after cutting was not affected by delaying application for three days.

There were reports on use of growth retardants to supplant mechanical mowing along roadsides (Freyman et al., 1986; Middlebrooks, 1986; Wakefield et al., 1986; Dickens, 1987; Burkhardt and Morré, 1986) but the need for brush control and additional research remains. The most cost effective mixtures are those based on basic work carried out under the Joint Highway Research Project at Purdue (Morré, 1982, 1983, 1984, 1985, 1988; Morré and Tautvydas, 1986; Freeborg et al., 1985).

According to a Summary Report published in 1988 (Morré, 1988), the Indiana Department of Highways is currently responsible for vegetation management on about 100,000 acres of roadside that receives at least one full cycle of mowing and includes other areas, such as medians and corridors adjacent to traffic lanes, that are mowed twice or three times. Both force-account and contract means are used at a total cost of $3,250,000/year. As an alternative to mechanical mowing, a program of chemical mowing was implemented for the state of Indiana in 1983 with 3 consecutive years of success with use on Interstate and dual-lane roads.

Background and Significance of Work.-- Effective use of chemicals in roadside management is tied at least in part to the availability of new materials through the efforts of the agricultural chemical industry. This includes materials potentially useful for the control of brush and other woody species along roadsides which is an essential maintenance operation.

The herbicide 2,4-5-T is probably the most effective known for control of brush. However, because of the possible presence of a highly toxic impurity in 2,4-5-T formulations known to cause birth defects, the use of this material has been discontinued. So far, a suitable single agent replacement for 2,4-5-T has not been developed. 2,4-D and related phenoxy-acid herbicides used widely for general weed control have only low to moderate activity against brush, and many woody species are resistant. The situation is even worse for Banvel and Telar which control very few woody species, although Banvel (dicamba) has proven effective against some species in combination with phenoxys. Tordon (picloram), while effective on brush, cannot be used in spring or summer adjacent to cropland due to the possibility of the material moving into soybean fields through surface water. Other brush materials such as Krenite also show a limited spectrum of species susceptibility when used as single agents.

The approach to be taken in the research proposed is to examine a spectrum of new materials (examples include chlopyralid, triclopyr, fluroxpyr, amidichlor, chlorflurenol, paclobutrazol, fluorprimidol and imazethapryr as well as the sulfonylureas (chlorsulfuron, metsulfuron and sulfometuron) alone and in various combinations with the phenoxy and other environmentally safe but presently inadequately effective potential brush control agents. The hope will be to generate a new cost-effective and environmentally safe mixture of materials effective against the majority of the woody species presently encountered along Indiana roadsides. Environmental safety of many of the newer materials will require evaluation as part of the work proposed. At the same time, the research will be carried out to combine the brush materials in such a way that it may be possible to achieve control of both brush and of milkweed and other 2,4-D-resistant species by a single mid-summer application. Additionally, different mixtures will be developed for application during different times of the year (e.g. late fall, early winter, early spring) in order to extend the application window and to permit some roadside vegetation maintenance operations to be moved to times other than the peak activity periods of mid- to late-spring and summer.

In work ongoing and scheduled for completion in the current fiscal year, considerable progress has been made in the development of total vegetation management chemical mixtures for use to control seed heads on secondary roads with mixed fescue, smooth brome and bluegrass stands. While not yet tested extensively under implementation conditions, results are sufficiently encouraging to warrant limited implementation testing of these mixtures under this project to determine implementation feasibility and evaluation prior to termination of the research program on chemical mowing. There is one application for these new growth retardant mixtures on secondary roads that came out of the discussions with District personnel that would be used and would greatly reduce mowing costs. This is for trimming around signs, posts, guard rails and other stationary objects located in areas to be mowed. Present procedure is to use Roundup. It is expensive, is unsightly, kills nearly everything and must be applied every year. With a 2-3 ft diameter circle around the post, hand trimming is largely eliminated but mower trimming is still needed. Mower operators are requesting 6-9 ft diameter areas in order to eliminate mower trimmings and not to have to slow down. With Roundup, these become unsightly and, especially at guard rails or on slopes, open up large areas to severe erosion. Better were the turf species not killed and a full season retardant material used. The application costs would be nearly the same, appearance would be improved, environmental safety would be significantly enhanced and savings would result from reduced mowing. This is definitely an area where the retardant work could be implemented and one where wide acceptance with substantial savings in cost might well be realized (some areas could be mowed in half the time). Other than the above mentioned implementation evaluation trials, no new work related to chemical mowing would be initiated.

3) Problem Statement

In 1971 a fall-spring spraying rotation for control of broad-leaf weeds was instituted in Indiana when approximately 1,500 linear miles of highway received a fall application of the environmentally safe 2,4-D amine under the spraying program by contract. The fall application was followed by a second application in early spring. In 1972 the program was expanded to a 3-year spraying rotation so that each year approximately 1/3 of the state was sprayed. The rationale of this program was that the treatment was, on the average, 90% effective in the control of broad-leaf weeds. With a weedy road having a weed density of 100,000 weeds per acre, the reduction is down to 10,000 weeds per acre which is quite acceptable in terms of appearance and maintenance of a healthy turf. Approximately 3 years were required for the weed population to reestablish to the point where a large return is obtained from the spraying cycle. In subsequent years, new materials such as Banvel and Telar have been included with the 2,4-D (Mason 1988) primarily to aid in control of wild carrot and the frequency of application has been increased to a spring application every other year. In the now more than 15 years since this program has been operational, general roadside weed problems have all but been eliminated. A reduction from 5-cycle to 3-cycle mowing resulted in substantial cost savings and in some years even further reductions in mowing have been possible.

A major emerging problem with a program involving spraying with 2,4-D amine, or even with the improved program using Banvel or 2,4-D amine plus Telar (Mason 1988) is the invasion of roadsides by brush, brambles, briars and trees. Brush and even small trees will kill turf by shading and competition which leaves patches of bare soil open to erosion. Trees close to the pavement represent a traffic hazard and must be removed. The turf must then be re-established to prevent further erosion. Even full-width mowing is not the answer. Trees and brush small enough to be mowed will not be killed but will continue to resprout year after year. The size of their root systems will increase until a sapling several feet high and more than an inch in diameter will be produced in a single growing season with some species. Additionally, many roadsides are already heavily infested with trees and brush that are too large to mow. This is especially prevalent in areas where the terrain prevents full cycle mowing (steep banks, cuts and fills, for example). Also, with wide medians and areas around interchanges, it may not be practical to mow full width every season. Further reductions in mowing are even more likely in the coming years. In discussions with Dan Zay, IDOH, the problem of brush control was judged currently to be a priority vegetation management problem for Indiana Roadsides. It is a problem that would be facilitated greatly by the availability of new treatment combinations that could be applied in the contract program and that would be amenable to spot application to prioritized areas and performance evaluation. Presently such combinations are not available and a number of new materials require evaluation to determine suitability for use in such a program.

According to the District Landscape Supervisors, the research was needed and the brush control materials would be used primarily in three different situations: 1) To prevent encroachment (for example, of black locust) on Interstate and 4-lane roads, 2) at bridges where brush is always a problem and 3) on insides of curves when brush begins to restrict sight distances. The primary use would be as a spot application and the proposed research was for development of combinations of materials for this type of use.

The primary need for research on brush control agents stems from the fact that presently no single material is available that will control all dominant roadside brush species. For example, Escort apparently is effective on Mulberry but does not control Box Elder. At present it is often necessary to spray the same area several times with different agents to control all of the brush present. The only known single agent that will give truly broad-spectrum brush control is 2,4-5-T which is no longer used. Therefore, mixtures of materials with the desired broad-spectrum characteristics need to be developed. this is information that is often difficult or impossible to get from Chemical Manufacturers, since several different manufacturers will be involved with each mixture. As a rule, best use of materials in combination is determined the University or some other independent agency, which has no particular product to sell.

Also to be developed would be combinations of materials to eradicate certain problem weeds, especially milkweeds and Canadian thistle, that are resistant to 2,4-D, Banvel and the 2,4-D plus Telar mixtures. Thistles occur in localized patches and are amenable to control by spot applications. Milkweeds, on the other hand, are a more general weed problem that is severe on some roads but not on others. There is no question that unsightly milkweed growth is removed by mechanical mowing but the plants are perennials and continue to come back in the same place every year. What is proposed would be a milkweed eradication program. One mid- or late-season application on those highway segments where milkweed seems to be taking over would be anticipated perhaps once every 5 years. Milkweed seeds germinate about as poorly as those of Canada Thistle so that, once controlled, reinfestation would be extremely slow.

While several aspect of vegetation management will be considered, the primary purpose of the research proposed is to develop new treatment combinations for use along Indiana roadsides primarily for spot control of brush. Combinations will be sought that are effective, environmentally safe and of low cost.

4) Objectives

This project was for research to develop and implement new treatment mixtures for control of problem brush, trees and other woody species and herbicide-resistant weeds along Indiana roadsides. Since single agents with the broad spectrum activity required for roadside use are presently unavailable, emphasis will be on the use of tank mixes developed for specific purposes based on identified needs. In addition, new materials as they become available were to have been evaluated and introduced into the mixtures and use situations to evaluate them as suitable replacement constituents that may be more effective, safer or less costly.

Specific objectives were as follows:

a) To prioritize within the safety zone what trees and brush are undesirable and in what areas. This was something that must be done on a road by road basis for Indiana and was requested as a top priority item to be carried out specifically to meet Indiana's needs.

b) To examine a wide spectrum of herbicide and growth retardant materials alone and in various combinations for control of brush. These are listed under d on the following page. Dominant roadside species will be identified and work will be directed toward the control of those species. Use of systemic (rather than soil sterilant) materials will be emphasized. Such an evaluation has not been done since about 1975 and there are many new materials that have come onto the market as well as experimental materials.

c) To develop different treatment combinations for different time of the year to include spot treatment by both foliage and bark applications. This will allow for an extension of the application window away from present peak periods to reduce costs by giving contractors greater flexibility in arranging their work loads. This is not information that industrial cooperation are ever likely to generate since different products from different manufacturers are to be combined as recommended mixtures.

d) To develop methods for rapid evaluation of brush control efficacy to facilitate performance payment of contract applications. This is work not done before and, in any event, would need to be developed for Indiana conditions. This was a specific request identified by landscape personnel of the IDOH.

Brush control chemicals to be examined alone and in combination. Several experimental materials (numbered compounds) would be available for testing as well.

Brush Control Agents

Common Name	Product Name
2,4-D	(Several available)
2,4-D ester	(Several available)
chlopyralid	
triclopyr	Garlon
triclopyr ester	Garlon-4
picloram	Tordon
fluoxypr	Starane
hexazinone	Velpar
dicamba	Banvel
glyphosate	Roundup
sulfometuron methyl	Oust/Escort
tebuthiuron	Spike 80W
fosamine ammonium	Krenite
dichlorprop	2,4-DP
fenoprop/silvex	Kuron/Weedone TP
tridiphane	Tandem
chlorsulfuron	Telar/Glean
imazapyr	Arsenal

e) To evaluate several newly introduced systemic herbicides in comparison to standard materials both for control of brush and other woody species and for spot control of noxious weeds such as Canadian Thistle and for Milkweed eradication where needed. The evaluation of herbicides for noxious weeds is an ongoing activity in a successful program. So many new materials reach the market each year that it is difficult to know which ones are best for Indiana unless an active program of evaluation under Indiana conditions is in place.

f) To continue a series of environmental safety trials in progress to monitor possible adverse effects of continuous herbicide applications and to initiate new trials as implementation of new materials or combinations of materials are anticipated. These environmental safety trials are critical. Some of these have been in place for several years, are under Indiana use conditions and are not being duplicated anywhere. It seems vital to the interest of chemical weed and brush control programs both in Indiana and nationwide that these trials be continued.

g) To follow through with a limited series of implementation trials to permit actual use evaluations of new combinations for grass seed head control, for use on secondary roads and in roadside chemical trimming operations around signposts and guard rails to reduce mowing costs. The implementation tests proposed concern as aspect of past work that is totally new and one where considerable cost saving may be possible. If the chemical trimming operations can be adapted to use new chemical mixtures developed under past research, the potential cost savings expected in the first year would more than pay for the cost of the entire research project proposed. This, certainly, is work not done anywhere else nor work that will likely be done elsewhere. The potential cost savings will not be realized without initiation of the proposed project.

h) To monitor all aspects of environmental safety associated with the use of chemicals and chemical combinations along Indiana roadsides such as carryover, drift, unfavorable interactions, damage to non-target vegetation, injury to fish, aquatic organisms, wildlife and man as well as any possible hazards to the applicator. The greatest limitation to evaluation of environmental safety is real data from actual use situations that gives new information upon which environmental decisions can be based. This is especially critical in the roadside management area where conditions and uses are sufficiently unique to warrant studies under exact conditions of use rather than to extrapolate from power company right-of-way studies or forestry use studies, for example.

All research was to be carried out in close consultation and cooperation with the Indiana Department of Highways and with representatives of the chemical manufacturers involved.

With the wide range of chemicals now available for use for roadside vegetation management, the assurance of environmental safety provided by this project remains as an important benefit. We would continue to ensure that only environmentally safe materials are used and that use of materials not environmentally safe (e.g. Weedone or other ester formulations) be discontinued. Since we started our program of environmental testing several years ago and began recommending only safe materials, public complaints and lawsuits over damaged crops, dead livestock, fish kills, personal injury, etc. due to roadside spraying have all but been eliminated.

Benefits.--Cost savings, safety and appearance are the primary benefits to be expected from the programs once implemented. Even with a return to full-width mowing, brush is an increasing problem of roadside management. In the past, the herbicide 2,4,5-T was used extensively and effectively as a brush control agent along roadsides. However, regulatory rulings now restrict its use. Therefore, a need for research to develop new treatment combinations for control of brush is a priority objective to aid roadside vegetation management.

According to Transportation Guides for Determination of Mowing Limits, safety overrides all other features affecting roadside maintenance. Sight distances must be maintained at intersections and on the insides of curves. Safety setbacks must be observed. Guard rails, bridge approaches, signs, and other traffic control devices must be kept open to view.

Brush is one of the major offenders in obstructing vision. Within two years black locust, willow or elm will become established even with new construction. In non-prairie areas, where woody vegetation is natural to the environment and a continual invader, one must be prepared to make periodic repeat applications even with efficacious mixtures. Tree seedings or root sprouts grow up into trees which represent solid objects and present even more serious safety hazards. Trees too near traffic lanes must be removed normally at considerable expense if mechanical means are used and the trees have been allowed to become large. Mechanical removal of established trees and of trees and brush growing in areas inaccessible to mowing is a very expensive alternative to application of brush-controlling chemicals.

In areas where brush and trees have still not become established to the point of requiring mechanical removal, the present approach to the prevention of further encroachment by woody species is full-width mechanical mowing. The State of Indiana is presently mowing full width at least once per growing season and some districts are considering full-width mowing at every mowing cycle largely because of the brush problem. The practices will more than double current mowing costs based on limited width 3-cycle mowing.

Mechanical mowing is presently the most expensive feature of roadside maintenance in Indiana. About 45,000 acres are mowed in the contract program with an additional 55,000 acres in force account mowing by State crews. With current cost estimates of $16.00 per acre per cycle for contract mowing and up to $30 to $35 per acre per cycle for force account mowing, costs are estimated to be between $2,000,000 and $3,000,000 annually with additional costs as more full-width mowing is added to the program. The present Indiana recommendations are one cycle of 3 in full-width so that slightly more than 50% of the mowing costs are in support of full-width mowing for control of brush. The availability of a good program of chemical brush control would virtually eliminate the need for full width mowing at every mowing cycle and reduce the need for full-width mowing overall. Through careful prioritization of what areas and what trees and brush in those areas would be treated, spraying costs will be kept at the minimum required to reduce or eliminate the requirements for full-width mechanical mowing.

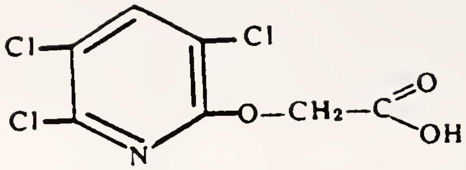
5. Work Plan and Analysis of Data

As summarized in the introduction, 2,4,5-T and 2,4,5-TP (Silvex) are effective as single agents for brush control but are no longer regarded as safe and the major objective of the work proposed was to develop a replacement material. The approach followed was to select a material chemically related to 2,4,5-T that was both safe and commercially available, to develop a theory for why the material was not as effective as 2,4,5-T and then to formulate a strategy to overcome the deficiency in the less-effective material.

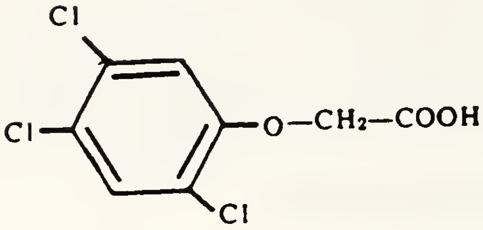
Based on preliminary screening, the material chosen for intensive study was Triclopyr (Garlon). The structure of triclopyr compared to 2,4,5-T and 2,4,5-TP is shown in Figure 1. Triclopyr is a distant cousin of 2,4,5-T and 2,4,5-TP and belongs to the same family of so-called auxin herbicides.

We then proceeded to conduct a series of laboratory trials comparing 2,4,5-T and triclopyr to determine what aspect of the mode of action of triclopyr was missing and to develop an inexpensive and environmentally safe means of restoring the missing activity.

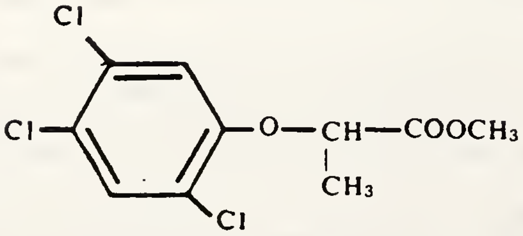
The model of growth control developed to guide these studies is diagrammed in Figure 2. What we visualize is an auxin-herbicide-mediated process involving a stimulated oxidation of reduced pyridine nucleotide (NADH)(Brightman et al. 1986). This is accompanied by a transmembrane flow of electrons and the creation of a diffusion potential that is negative at the outside of the stimulated cells. Also involved is an apparent requirement of chemical energy in the form of ATP. Based on laboratory studies, this requirement was postulated to be mediated via a "valosin-containing" type of ATPase.



Garlon



2,4,5-T



2,4,5-TP

Figure 1. The chemical structure of triclopyr (Garlon Herbicide) compared to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-TP (Silvex). The purpose of the study was to modify the action of triclopyr to enhance its level of brush control activity to that exhibited normally by 2,4,5-T and 2,4,5-TP.

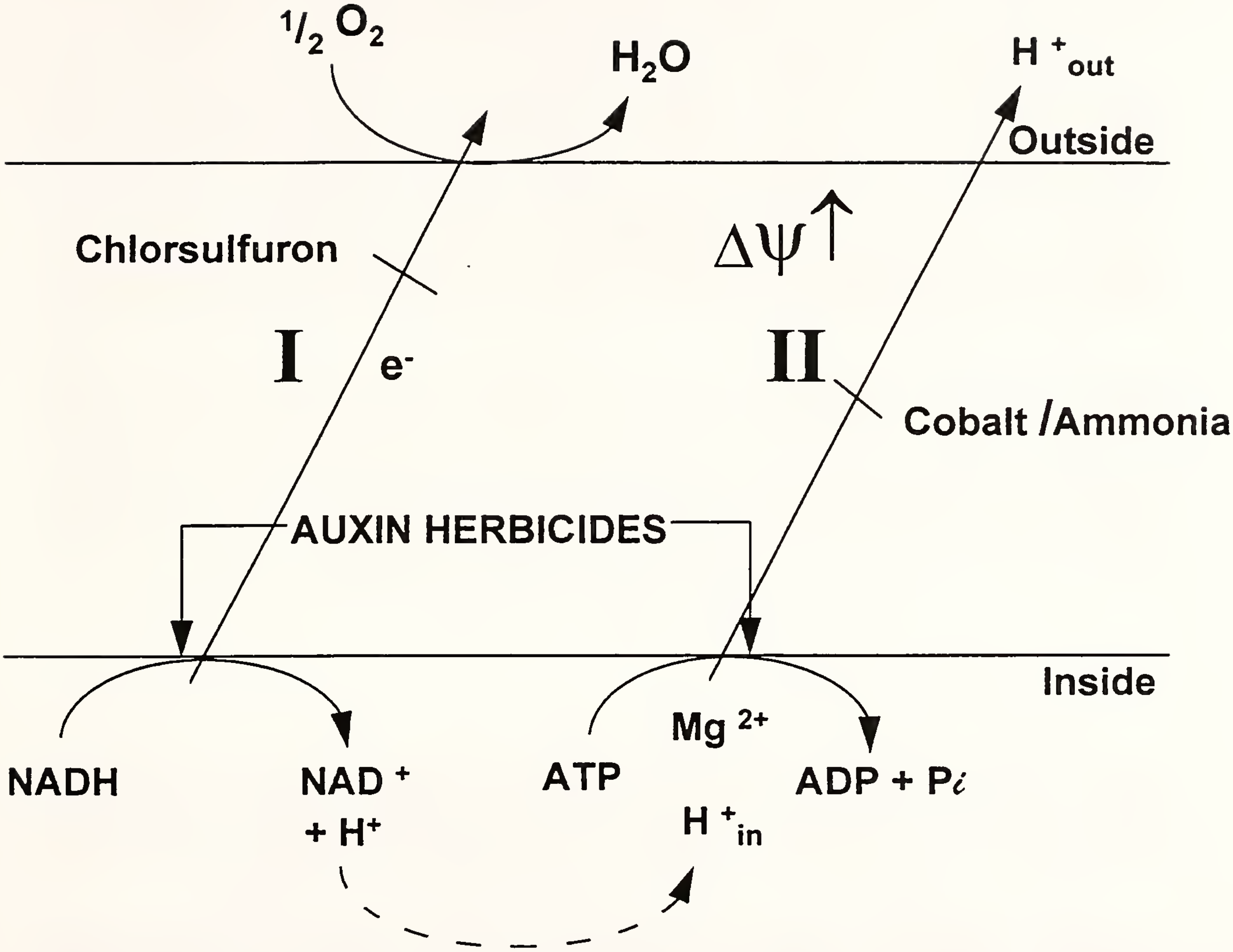


Figure 2. Simple model to illustrate the two-site concept of action of the auxin herbicides used for design of a brush-control strategy.

Both 2,4,5-T (2,4-D) and triclopyr were found to be of equal effectiveness in the electron flow part of the model (I of Figure 2). In these and other experiments, 2,4-D eventually was substituted for 2,4,5-T in the laboratory and greenhouse for safety and convenience. Since triclopyr appeared to be effective in the initial part of the scheme, we reasoned that the deficiency might reside further down stream at the postulated ATP-dependent step (II of Figure 2).

Assuming that the ATPase involved would be similar to that of the ATPase of vesicle budding (involved in physical membrane displacements), then it would exhibit properties similar to those observed for valosin-containing ATPases summarized in Table 1. It would be resistant to most of the inhibitors of other types of ATPases listed in Table 2. Auxin herbicides effects were found by experiment to be refractory to nitrate, vanadate, ouabain and oligomycin, inhibitors of vacuolar, plasma membrane and mitochondrial ATPases, respectively. In contrast, the ATPase was markedly inhibited by cobalt chloride, presumably by competition with magnesium or manganese ions which are required for activity.

Based on the above summarized rationale and the results summarized in Tables 1 and 2, a series of greenhouse, field and implementation trials resulted in the use of cobalt as an additive in combination with triclopyr (Garlon) for control of roadside brush. It was eventually shown that any cation capable of competing with magnesium or manganese but ineffective of itself would have the same effect. The ammonium ion was finally selected as the ion of choice. The effect of the competing ions was not to facilitate penetration of herbicide but to enhance the activity of the herbicide according to the model of Figure 2. These results are summarized in the sections of the report that immediately follow.

1. Cobalt and ammonium ions enhance the herbicidal activity of triclopyr

a. Laboratory studies

Laboratory studies were with dark-grown seedlings of soybean. Seeds were soaked in water and planted in moist vermiculite. After 4 to 5 days in darkness, segments were cut from the hypocotyls (stems) about 0.5 cm below the point of attachment of the cotyledons. Segments 1 cm long were used for growth studies and segments 2 cm long were used for isolation of plasma membranes.

Plasma membranes were isolated from an 8,000 X g to 50,000 X g pellet (microsomal membrane fraction) prepared by centrifugation of homogenates (hypocotyl segments ground in an isotonic solution at physiological pH). The isolation of plasma membrane was by aqueous two phase partition in which polymer mixtures of dextran and polyethylene glycol were used that spontaneously separate into two phases. The resuspended crude membranes were mixed together with a 6.4% phase system of the two polymers above. After mixing, the two phases were separated by centrifugation and the plasma membrane vesicles partitioned into the upper phase. The isolated plasma membrane vesicles were frozen and stored frozen at -70 C until use in an experiment.

NADH oxidase activity was determined using a DW2000 spectrophotometer (SLM-Aminco) in the dual-wavelength mode as the decrease in A340 with A430 as reference. Rates were recorded with an attached HP plotter/printer. Reactions contained 20 mM Kris-Mes, pH 7, 150 μM NADH and 1 mM KCN. Assays were at 25°C

Table 1. Properties of the proton ATPase and TER ATPASE

	P	F	V	TER ATPase
pH optimum	6.5	8.0	7.0	8.5
Ion stimulation	K^+	-	CL^-	-
Inhibitors	VO_4^- DCCD	Azide DCCD Oligomycin	NO_3^- DCCD NEM	Co^{2+} Azide

P-ATPase (E_1-E_2 type) encompass a variety of cation-specific pumps such as the Na^+/K^+ - ATPase of the plasma membrane and the Ca^{2+} -ATPase of the sarcoplasmic reticulum.

F-ATPase (or F_o-F_1 type) are present on the inner membranes of mitochondria and chloroplasts.

V-ATPase (or vacuolar type) are present on the endomembrane system including vacuoles, lysosomes, endosomes, Golgi apparatus, chromaffin granules and coated vesicles.

Table 2. **Effect of ions and ATPase inhibitors on TER ATPase activity**

Addition	Concentration	ATPase activity % of control
Control		100
Ions		
- $MgCl_2$		14
+ $CaCl_2$, - $MgCl_2$	2 mM	105
+ $CaCl_2$	10 mM	58
ATPase inhibitors		
Vanadate	50 μM	102
KNO_3	50 mM	92
Ouabain	1 mM	85
Oligomycin	10 μg/ml	78
NaN_3	1 mM	76
NEM (10 min, 37°C)	1 mM	56
ATP-γ-S	2 mM	35
$CoCl_2$	10 mM	7

Control reaction contained 0.1 mM NaN_3, 1 mM sodium molybdate, 2 mM $MgCl_2$, 2 mM ATP, pH 8.2. The reaction was at 37°C for 1 h.

for 5 to 10 min with constant stirring. Unless indicated otherwise, results are the average of two determinations from either three or two separate experiments + standard deviations (n = 3) or mean average (n = 2) deviations. Proteins were measured by the bicinchonninic acid procedure. Plasma membranes (50-100 μg) was added to start the reactions.

For electron microscopy, the isolated vesicles were separated into right side-out and inside-out vesicles by preparative free-flow electrophoresis and then incubated with or without auxin herbicide (2,4-D, triclopyr or picloram) and either ATP (a source of chemical energy), NADH (a source of reducing power = electrons and protons and/or chemical energy) or both. The vesicles were then fixed by addition of 2.5% glutaraldehyde (diluted 1:10) with the suspended membranes. The vesicles were then collected by centrifugation and fresh 2.5% glutaraldehyde was added. The pellets were dehydrated through an acetone series and embedded in plastic (Epon). Thin sections were cut with an ultramicrotome, the sections were surface-stained with lead, and the sections were examined and photographed with a Philips EM200 electron microscope. Vesicles diameters were measured using a ruler from prints magnified 35,000 times.

For growth studies, 1 cm hypocotyl segments cut as described above were floated on water solutions (unbuffered) with or without the auxin herbicide or other additives. Segment length after 12 to 18 hours of growth was measured to the nearest 0.05 cm using a ruler.

i) Cell-free physical membrane displacement using the electron microscope for visualization and measurement.

The concept of the experiment is illustrated in Figure 3. Normally the herbicidal action of 2,4-D, triclopyr, picloram and other auxin herbicides involves cell enlargement (top two rectangles). This process is energy-dependent as indicated for the conversion of a source of chemical energy, ATP, into its less energy-rich ADP form. The ATP-driven enlargement involves a process we have termed physical membrane displacement. This means that the membrane which surrounds the elongating cells becomes physically displaced to increase its surface.

To study this in the laboratory, the cells diagrammed as membrane-enclosed rectangles were broken. The surrounding membrane forms spheres represented by the circles in the second row of the diagram. Some of the spheres form right side-out with the arrows on the inside (the site of ATP utilization) and some of the spheres form inside-out with the arrows on the outside. These two populations of vesicles were then resolved by preparative free-flow electrophoresis into separate fractions enriched in inside-out vesicles with the ATP site on the inside (3rd row) or right side-out vesicles with the ATP site on the outside (last row). The two populations of vesicles were then incubated with ATP and NADH to energize physical membrane displacement stimulated by the auxin herbicide, 2,4-D. The 2,4-D can penetrate both types of vesicles but the NADH and ATP cannot. They will work only with the vesicles that are inside-out with the ATP and NADH sites on the inside of the cell exposed. Such vesicles, when treated with ATP, NADH and 2,4-D actually increased in diameter and volume.

The distribution of vesicles is shown in Figure 4. On average the control

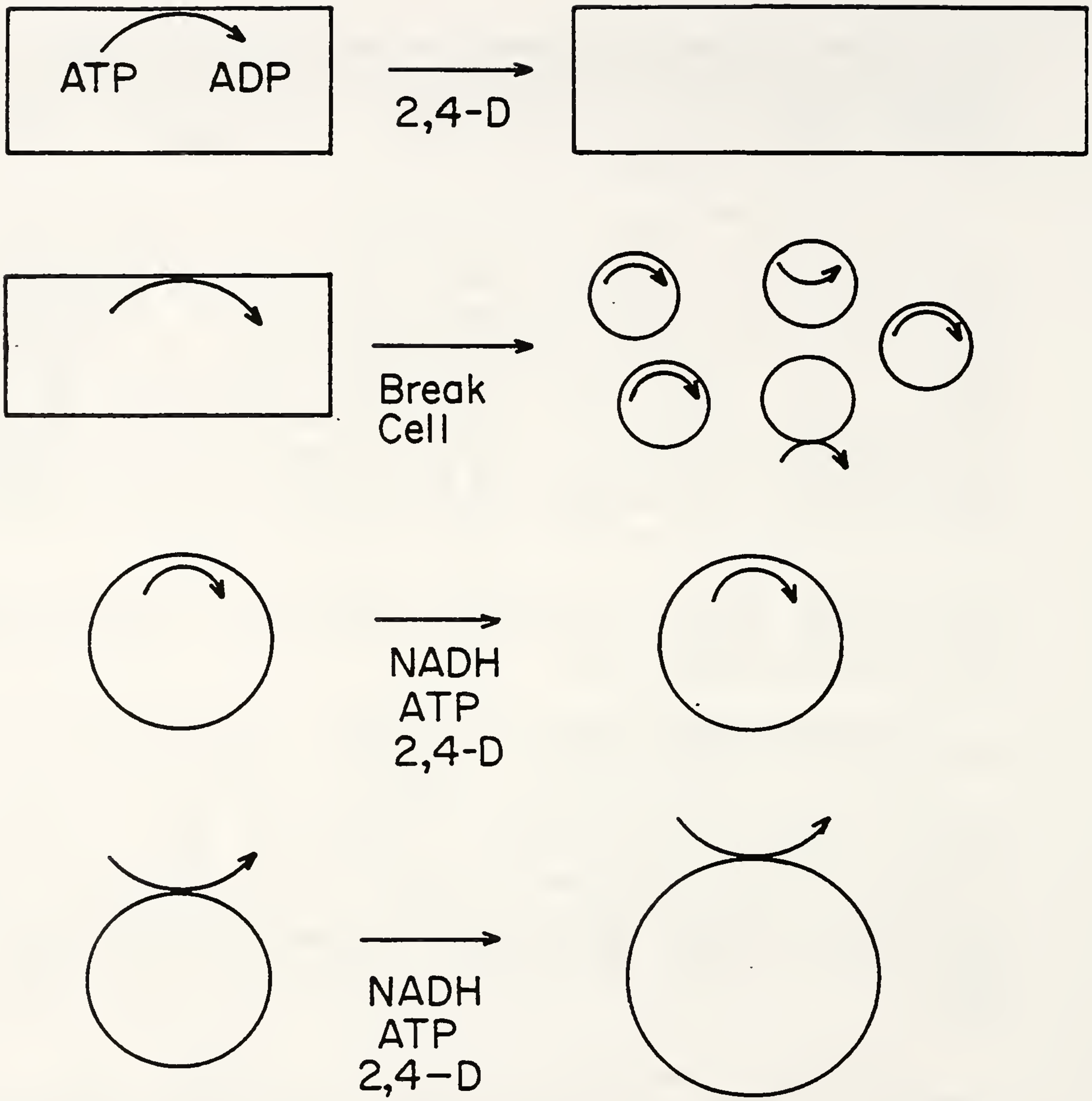


Figure 3. Experiments using electron microscopy to recreate a physical membrane displacement reaction stimulated by auxin herbicides in the laboratory. In the top row, plant cells diagrammed as rectangles are stimulated to enlarge by the auxin herbicide, 2,4-D. The process is driven by energy from ATP but the ATP site is on the inside of the cell. In order for ATP from the outside to reach that site, the cells (rectangles) must be broken (second line). Once broken, the membranes of the cell form spheres. Some of the spheres are inside-out with the ATP site exposed. Others are formed right side-out with the ATP site hidden. The inside-out vesicles respond to an energy source (ATP) in the presence of a source of reducing power (NADH) and the herbicide by increasing in diameter (bottom row). The right side-out vesicles do not respond because the ATP and NADH which are needed for the process cannot enter (next to last row). 2,4-D can penetrate the vesicles and is active from either side. The herbicide-stimulated vesicle enlargement was used as an assay as illustrated in Figures 4 and 5.

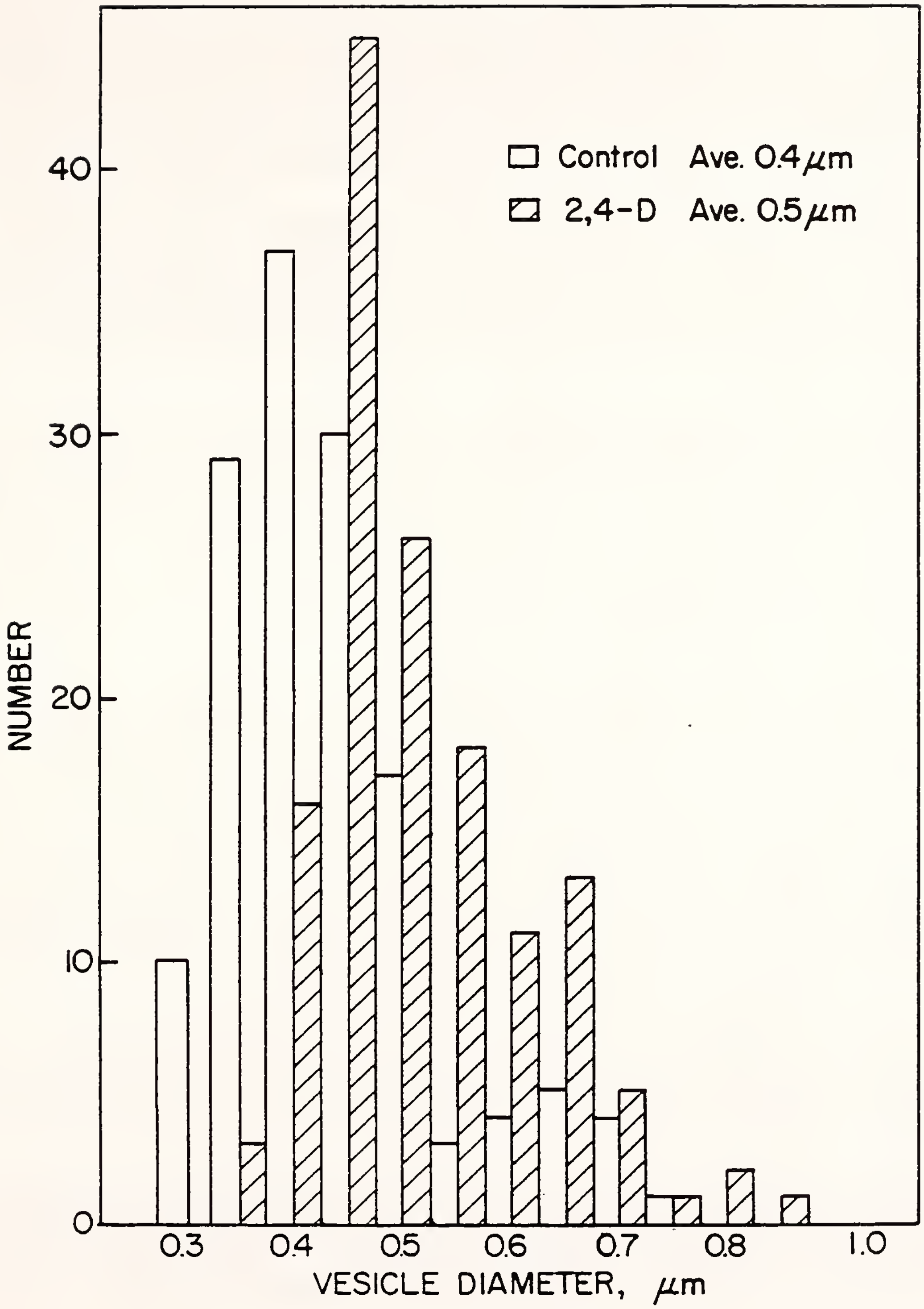


Figure 4. Quantitation of the response of inside-out plasma membrane vesicles from soybean incubated in the presence of ATP and NADH to the auxin herbicide 2,4-D. Vesicle diameters are shifted in distribution toward larger diameters as a measure of vesicle enlargement.

vesicles were 0.4 μm in diameter whereas those treated with 2,4-D were 0.5 μm in diameter. The average calculated volume of the spherical vesicles of the control preparation was 0.007 μ^3. The corresponding calculated volume for the preparation with 2,4-D was 0.016 μ^3, an increase of more than 2-fold. Although the distribution of vesicle diameters in the two preparations overlapped (Fig. 4), the vesicles treated with 2,4-D clearly were shifted to larger diameters.

With the inside-out vesicles, enzymes and proteins of the cytoplasmic aspect of the plasma membrane are accessible to external additions whereas the right side-out (cytoplasmic side-in) vesicles served as specificity controls. The doubling in volume over 30 min was seen only with cytoplasmic side-out vesicles. ATP was absolutely required for the response. Cytoplasmic side-in vesicles showed no response.

When the three auxin herbicides, 2,4-D, triclopyr and picloram were compared (Figure 5), the response in the electron microscope assay for physical membrane displacements were similar for each. The distribution of vesicle diameters and increase in vesicle volume was shifted in the presence of the herbicides.

All three herbicides stimulated cell enlargement (Fig. 6) and were tested in the electron microscope assay at concentrations of 10 μM which was near optimal for cell enlargement *in situ*.

The requirement for NADH in the physical displacement of membranes resulting from treatment with the auxin herbicides can be understood on the basis of the stimulation of a transmembrane flow of electrons from NADH to oxygen. The enzyme system responsible for this electron flow has been termed NADH oxidase II and is stimulated by 2,4-D and other auxins in a concentration-dependent manner. The 2,4-D stimulation is seen with isolated preparations containing the vesicles of plasma membrane and does not require the intact cell to be operational. This is one reason why we were able to carry out the cell-free vesicle enlargement reactions stimulated by 2,4-D of figures 4 and 5. The stimulation of NADH oxidase by 2,4-D compared to picloram and triclopyr is given in Table 3. All three herbicides were approximately equivalent in their stimulation of this activity at a near optimum concentration of 10 μM.

In order to augment the action of triclopyr as a brush control agent, two approaches were utilized. In the first approach, an inhibitor of the auxin herbicide-stimulated oxidation of NADH was sought. In the second approach, an inhibitor of the ATP requirement was sought.

To inhibit the auxin-stimulated oxidation of NADH, we initially employed the herbicide chlorsulfuron (Telar)(Fig. 7). Chlorsulfuron at a concentration of 1 μM inhibited specifically, the 2,4-D induced physical membrane displacement (elongation) of excised segments of soybean. In the absence of 2,4-D, the chlorsulfuron by itself was without effect on growth (Fig. 8).

Similarly, chlorsulfuron by itself did not inhibit the normally auxin-stimulated NADH oxidase activity of plasma membrane vesicles isolated from hypocotyls of soybean (Fig. 8). The upper curve of Figure 8 shows the stimulation of NADH oxidase activity by the addition of 2,4-D. What is being measured is the disappearance of NADH as a drop in absorbance at 340 nm (an

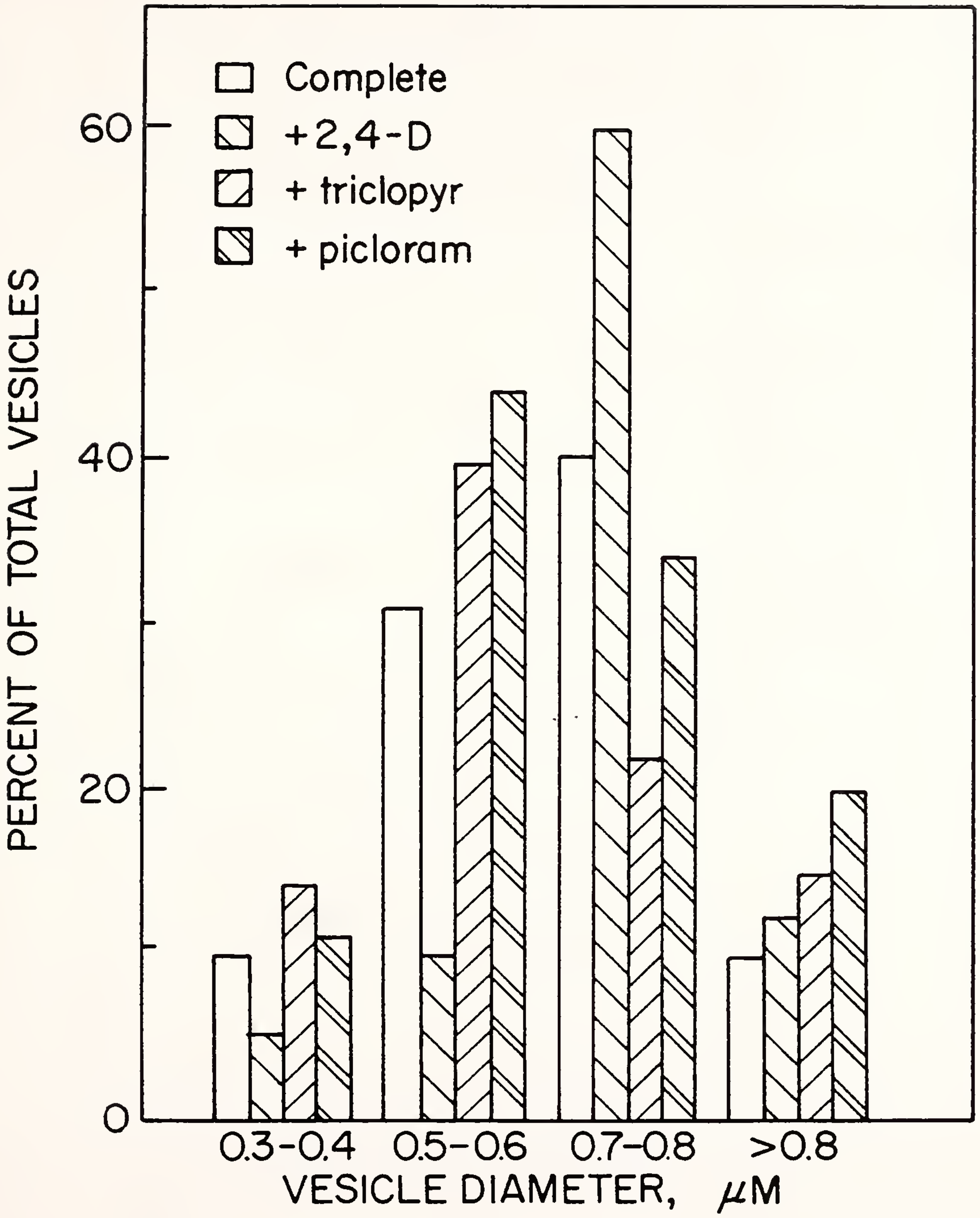


Figure 5. Distribution of diameters of vesicles of soybean comparing the response to three auxin herbicides, 2,4-D, triclopyr and picloram. For each, the auxin herbicide, when incubated in the presence of ATP and NADH, cause the cytoplasmic side-out vesicles to enlarge.

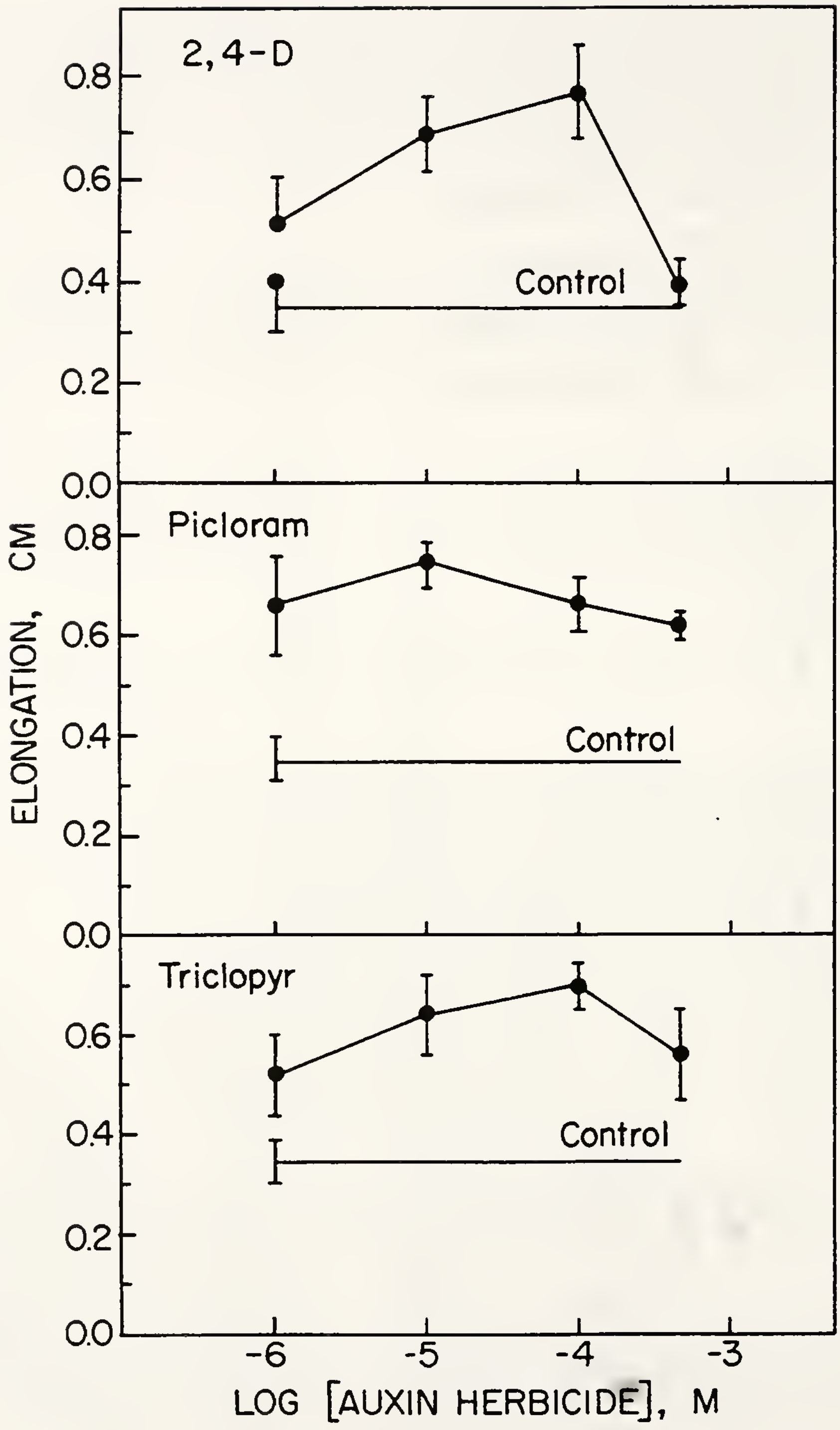


Figure 6. Growth response of 1 cm sections cut 0.5 cm below the apical hook of dark grown seedlings of soybean (the same plant material as used for the experiments of Figures 4 and 5). All three auxin herbicides stimulate physical membrane displacement (cell enlargement) with living cells shown here as well as with the isolated vesicles shown in Figures 4 and 5.

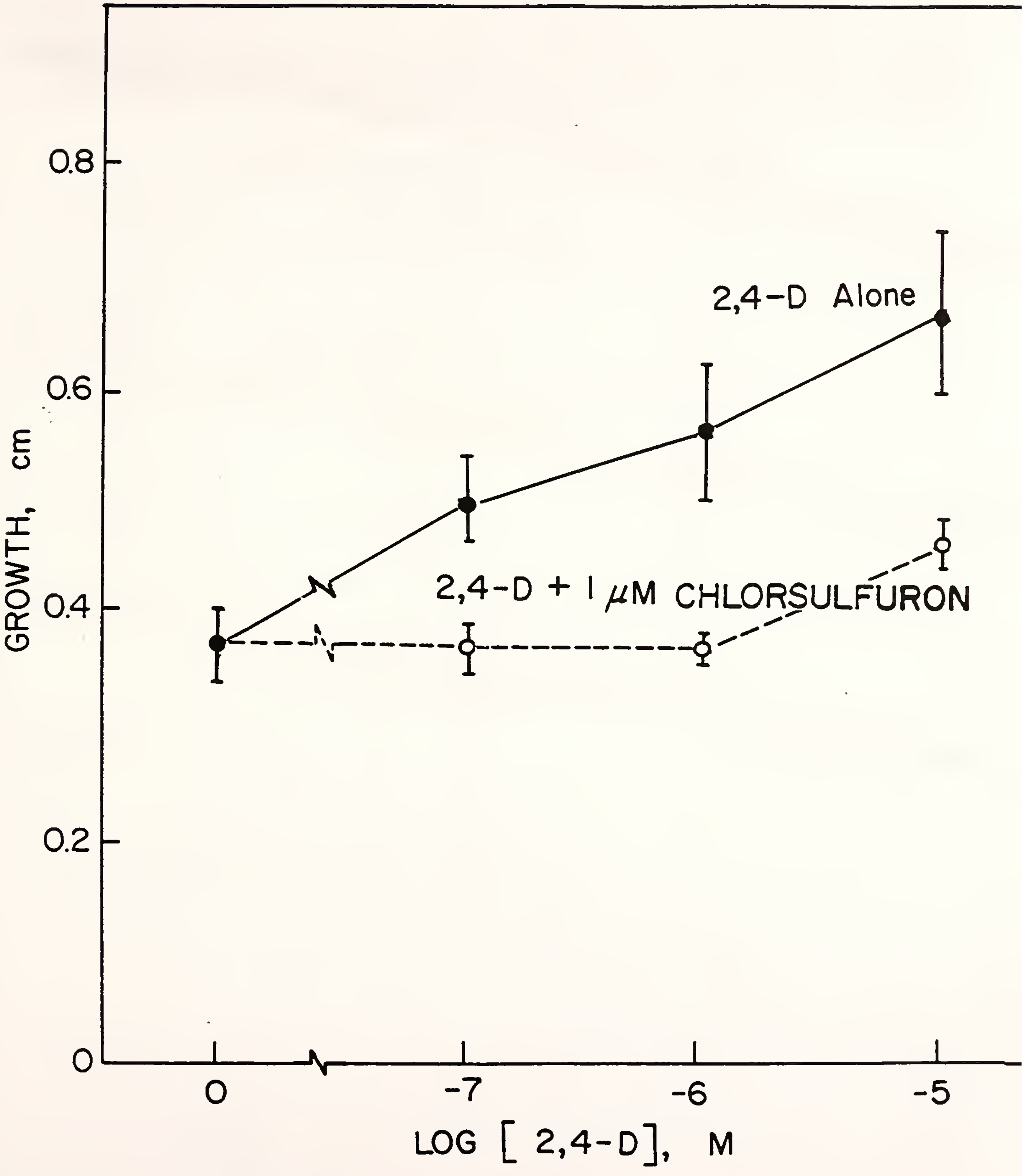


Figure 7. Stimulation of growth of 1 cm segments cut from dark-grown soybean seedlings in response to increasing concentrations of the auxin herbicide, 2,4-D and its inhibition by the presence of the sulfonylurea herbicide, chlorsulfuron (Telar). NOte that only the 2,4-D stimulated growth is inhibited.

Table 3. Stimulation of the oxidation of NADH by isolated vesicles from dark-grown stems of soybean by the auxin herbicides 2,4-D, triclopyr and picloram. This stimulation served as one basis for the development of the new brush-control additive mixtures which are the basis for this report.

NADH OXIDASE ACTIVITY OF SOYBEAN PLASMA MEMBRANES

Herbicide	Conc. M	nmoles/min/mg	% of control
None	-	0.6	100
2,4-D	10^{-4}	0.9	150
	10^{-5}	1.13 ± 0.005	188
	10^{-6}	0.88	146
Triclopyr	10^{-4}	0.76	124
	10^{-5}	1.05	147
	10^{-6}	0.98	163
Picloram	10^{-4}	0.84	140
	10^{-5}	1.20 ± 0.08	200
	10^{-6}	0.86	144

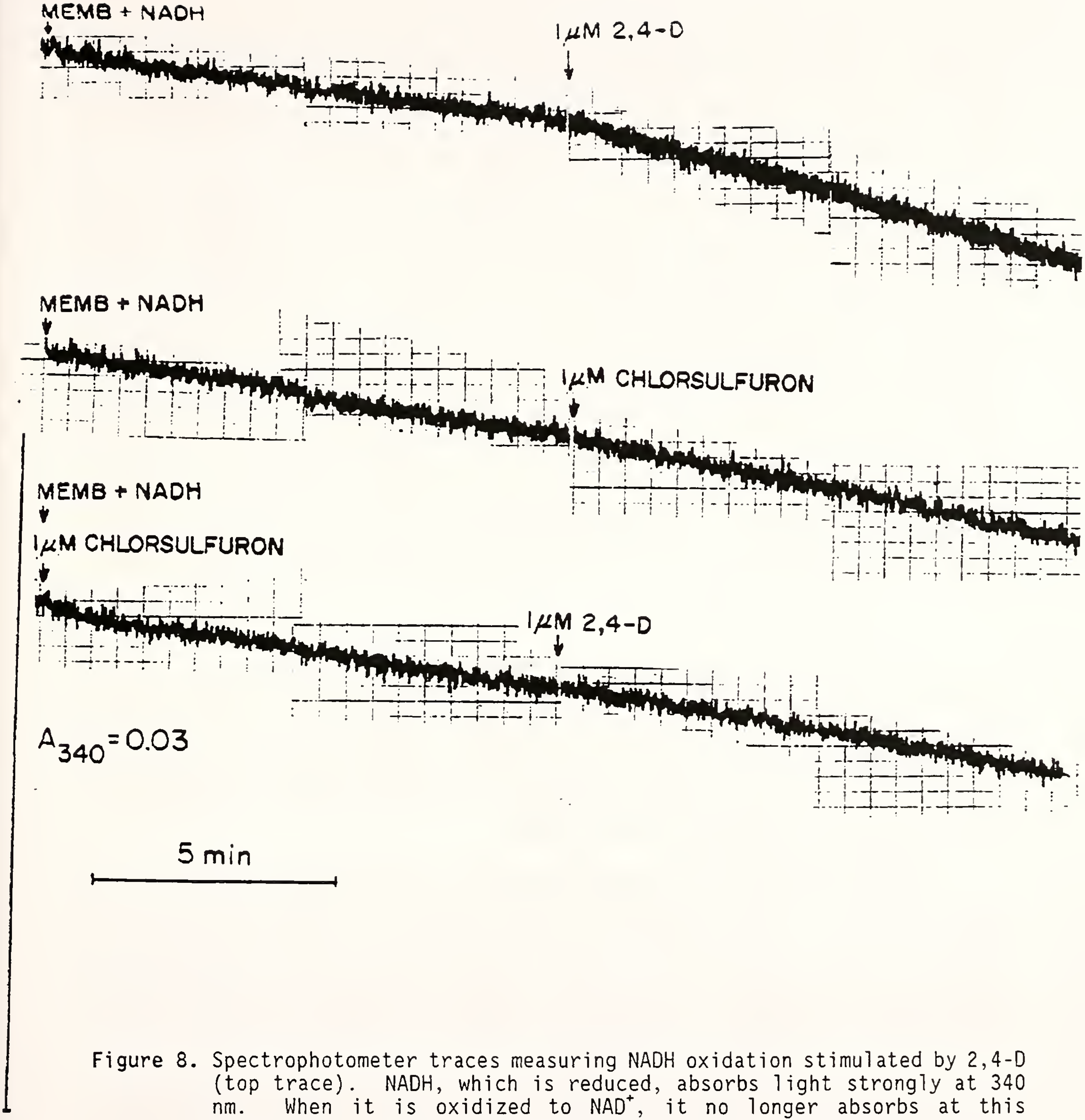


Figure 8. Spectrophotometer traces measuring NADH oxidation stimulated by 2,4-D (top trace). NADH, which is reduced, absorbs light strongly at 340 nm. When it is oxidized to NAD^+, it no longer absorbs at this wavelength. Therefore the drop in absorbance is a measure of NADH oxidation. The source of the oxidation activity is vesicles of plasma membrane prepared from dark-grown seedlings of soybean. Chlorosulfuron, by itself, is without effect or slightly stimulatory as well to NADH oxidation (middle trace). However, pretreatment of the membranes with chlorsulfuron (bottom trace) effectively prevents the stimulation of NADH oxidation by the 2,4-D herbicide.

increased downward slope). The middle curve shows the effect of chlorsulfuron by itself. The rate is accelerated slightly. The bottom curve shows the effect of chlorsulfuron on the response to 2,4-D. In the presence of chlorsulfuron, 2,4-D is unable to further stimulate the NADH oxidase. Thus, chlorsulfuron or Telar was selected as the material to combine with triclopyr to enhance its activity through the NADH oxidation part of the pathway (Fig. 2).

For the ATP part of the pathway, we examined the series of ATPase inhibitors listed in Table 2. Of these, only one, cobalt chloride was able to inhibit auxin-induced growth (Fig. 9), despite the fact that the other ATPase inhibitors such as nitrate and vanadate inhibited up to 50% of the ATPase activity of the plasma membrane. We have evidence for a magnesium- or manganese-requiring nitrate- and vanadate-resistant ATPase activity associated with the plant plasma membrane. It is this activity which we assume is related to the ATP-requirement for membrane displacement and which can then be inhibited by cobalt chloride to result in the inhibition of auxin-induced membrane displacement (enlargement) illustrated in Figure 9. The hypothesis is that the cobalt inhibition is the result of a competition of the cobalt ions with the magnesium and/or manganese ions required for activity by the ATPase involved in the herbicidal action of triclopyr. In any event, based on the results of Figure 9 and the fact that none of the other inhibitors tested had any effect on the auxin-induced elongation response, cobalt chloride was selected as the compound for initial testing in the greenhouse as the inhibitor of the second part of the pathway to restore some of the activity associated with 2,4,5-T to triclopyr (Fig. 10).

b. Greenhouse studies

For greenhouse studies, seeds of garden bean (*Phaseolus vulgaris* var.) were planted, five per pot, 1 inch deep in soil contained in 4 inch diameter plastic pots. The pots were placed in the greenhouse for germination and growth. After 7 to 9 days at about the time the first trifoliate leaf was expanding, the plants were treated by aerosol spray with or without triclopyr in the presence and absence of cobalt chloride or chlorsulfuron. To measure response to triclopyr, the curvature of the stem, a typical response to auxin herbicides, was measured, usually between 24 and 35 h after treatment.

As predicted from the laboratory studies, the herbicidal response of the bean plants to triclopyr was enhanced by the addition of cobalt chloride to the mixture (Table 4). The appearance of the plants is illustrated in Figure 11. Cobalt chloride by itself (second plant from the left) was without effect. The effect of cobalt chloride also was seen in the herbicidal activity with death of the plant as an endpoint (Figure 12). The plants treated with triclopyr plus cobalt chloride were all dead (extreme right) when plants in the pots treated with triclopyr alone were still alive.

In a series of experiments not illustrated, the optimum concentration of triclopyr to induce the bending response and to result in death of the plants in one to two weeks was determined to be 1/16 lb/acre. Triclopyr at 1/8 lb/A or higher tended to kill the bean plants too rapidly to utilize the greenhouse assay as a means to test for synergism between the triclopyr and the additives. Triclopyr at 1/32 lb/acre produced only a weak and variable herbicidal response. Therefore, for the greenhouse studies, the rate of triclopyr was fixed at 1/16

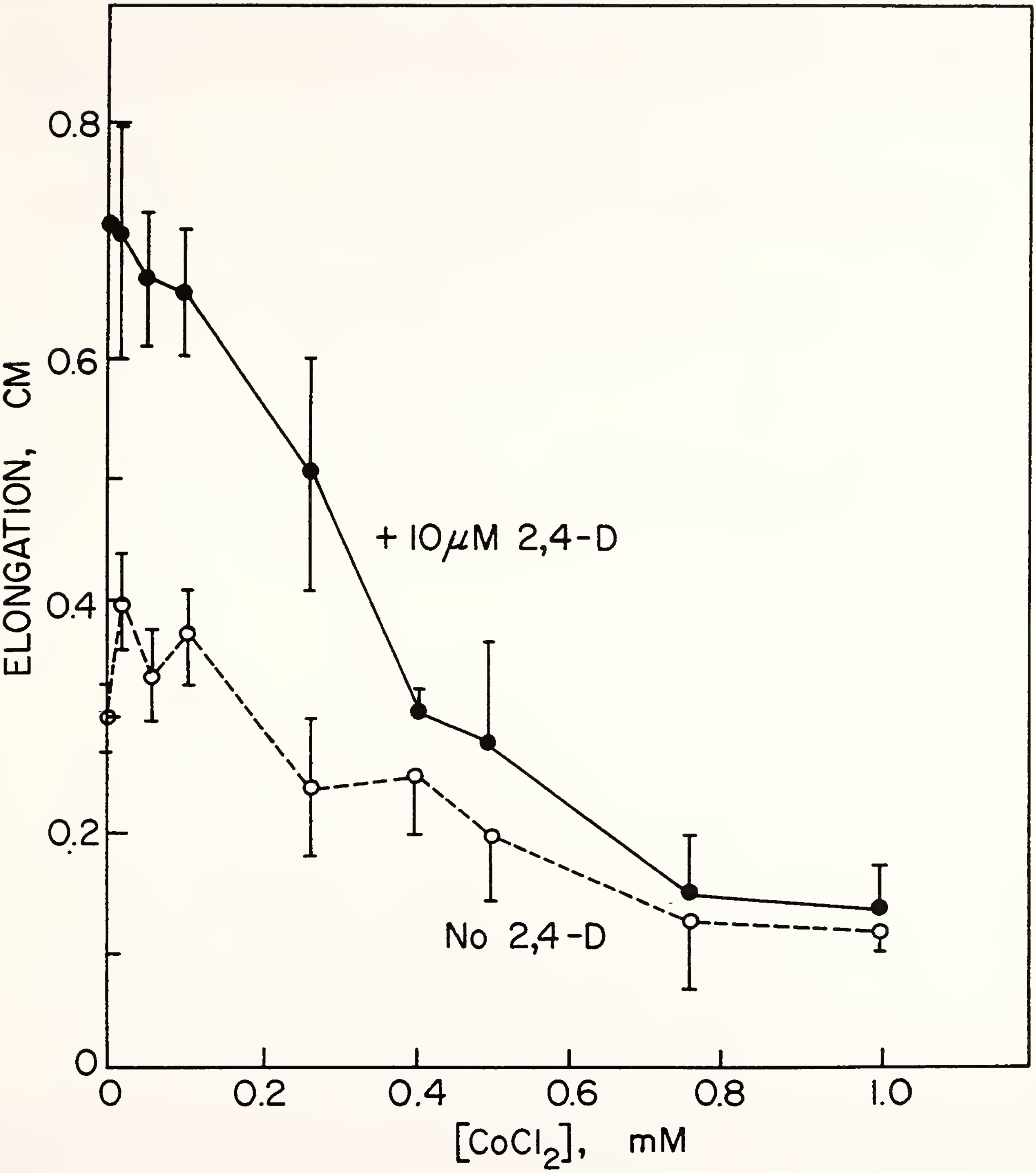


Figure 9. Inhibition of cell enlargement (physical membrane displacement) in 1 cm long segments of the elongating zone of dark-grown seedlings of soybean by increasing concentrations of cobalt chloride. At a concentration of 1 mM the cobalt chloride has completely inhibited the 2,4-D-induced growth but some growth in the absence of 2,4-D remains.

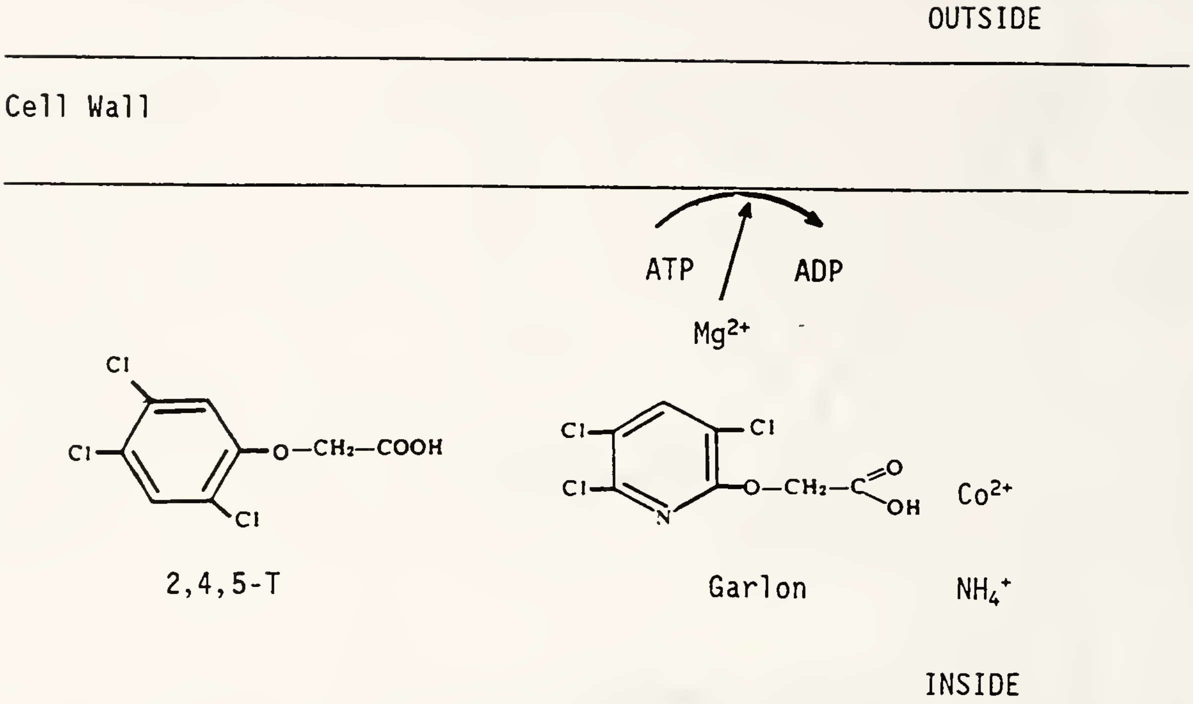


Figure 10. Model to explain the second part of the approach to enhance the herbicidal activity of Garlon (triclopyr) to a level of that of 2,4,5-T. In this approach, we have used cobalt ions (Co^{2+}) or ammonium ions (NH_4^+) to block the utilization of ATP in driving the energy requiring steps of physical membrane displacement stimulated by these herbicides at low concentrations and inhibited by these same herbicides at high concentrations (see Figure 6). The basis for the action of the cobalt and ammonium ions is thought to be to compete for the magnesium ions (Mg^{2+}) normally required for the energy utilization step of ATP hydrolysis (Table 2B).

Table 4. Response of garden bean in the greenhouse to trichlopyr (Garlon) in the presence or absence of the cobalt chloride additive. Results are from six treatment groups ± standard deviations. Evaluations were 3 to 5 days after treatment.

Trichlopyr	Cobalt choride	Stem curvature, Degrees
None	None	0 ± 0^a
None	10 oz/A	0 ± 0^a
1/16 lb/A	None	97 ± 12^b
1/16 lb/A	10 oz/A	136 ± 11^c

Numbers not followed by the same letter are significantly different ($p < 0.05$).

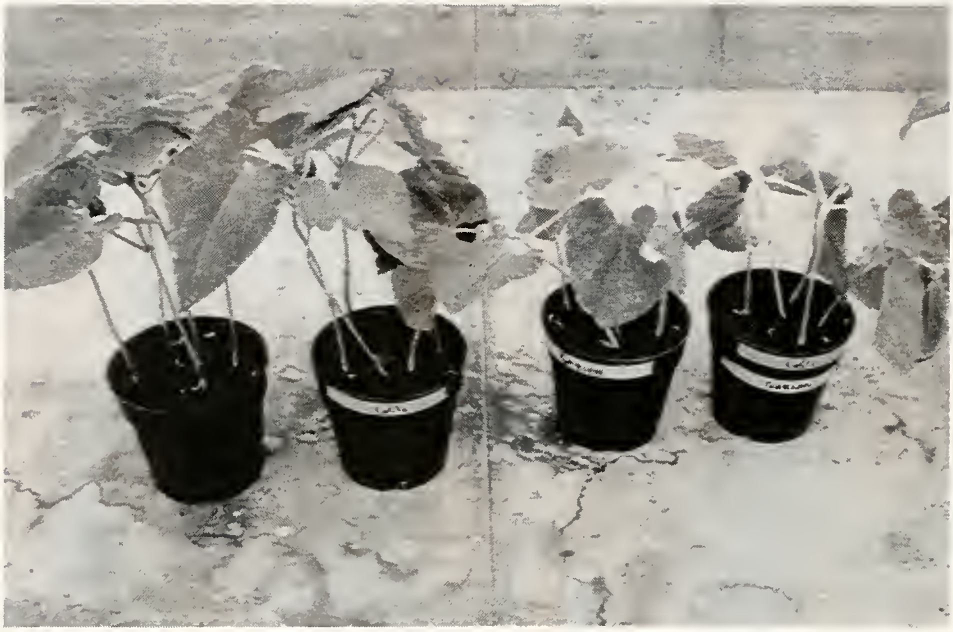


Figure 11. Garden bean 3 days after treatment. From left to right. Control, 10 oz/A cobalt chloride, 1/16 lb/A triclopyr, 10 oz/A cobalt chloride plus 1/16 lb/A triclopyr.

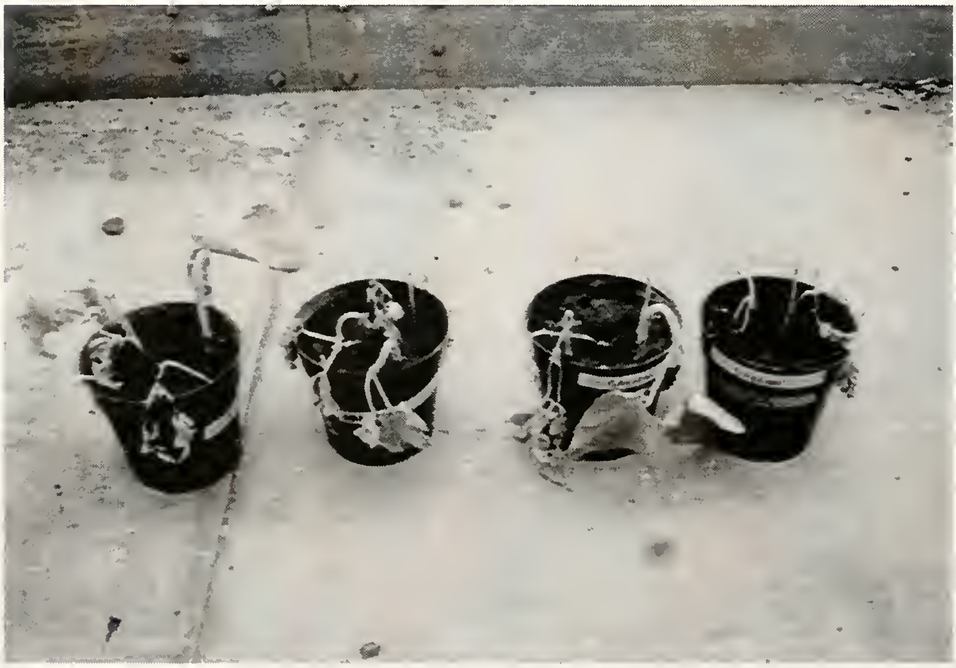


Figure 12. Garden bean 14 days after treatment. From left to right, the first three pots were treated with 1/16 lb/A triclopyr. The pot on the right was treated with 1/16 lb/A triclopyr plus 10 oz/A of cobalt chloride. With the mixture, all plants were dead whereas with triclopyr alone approximately 50% of the plants were still alive.

lb (1 oz)/acre.

In the presence of 1/16 lb/A of triclopyr, the concentration of cobalt chloride was varied over the range 0 to 10 oz/acre. A near optimum response was obtained at 10 oz/acre (Figs. 13 and 14).

The greenhouse evaluation of chlorsulfuron plus triclopyr revealed no consistent beneficial interaction of the two materials in combination (Table 5). Herbicidal activity of triclopyr on bean plants in these experiments was enhanced by cobalt chloride as in Table 4. However, a significant additional enhancement was seen in the three-way mixture of triclopyr, chlorsulfuron and cobalt chloride (Table 5). The enhancement of the herbicidal activity of triclopyr by the cobalt chloride additive was seen both in the presence and absence of chlorsulfuron at 10 oz of cobalt chloride per acre (Fig. 15) and in a dose response determination over the range 0 to 5 oz/acre of cobalt chloride (Fig. 16).

While cobalt chloride is a very effective additive in the mixture, it was neither inexpensive nor without possible environmental problems. Therefore a less expensive substitute was sought, the rationale being that any cation that would compete with magnesium or manganese in the ATP part of the pathway (Fig. 10) might be able to replace cobalt as an additive. On this basis, ammonium nitrate was tested in the greenhouse.

Ammonium nitrate at an application rate of 5 to 20 ounces per acre was equivalent to cobalt chloride at 5 oz/acre in enhancing the herbicidal activity of triclopyr on plants of garden bean in the greenhouse (Table 6). In addition, the three-way interaction between triclopyr, chlorsulfuron and cation also was seen at 20 oz/acre of ammonium nitrate (Table 6, last entry). Ammonium nitrate is readily available, environmentally safe, without hazards to man or animals and extremely inexpensive (costing less than $1.00 per 100 gallons of spray mixture).

Based on the laboratory and greenhouse studies outlined in this section of the report, triclopyr in combination with chlorsulfuron and either cobalt chloride or ammonium nitrate were taken to the field for testing in the control of brush under roadside conditions.

2. Evaluation of a foliage application of triclopyr plus additives for control of brush along roadsides.

The mixture of triclopyr plus cationic additives (cobalt chloride or ammonium nitrate) was first field tested in the third quarter of 1990 (August 27). The material was applied in water solution using a hand-held sprayer to the point of run-off. The brush was located in a fence line along IN 426 in Tippecanoe County and consisted of mulberry, elm, walnut and poison ivy. Triclopyr was applied as Garlon 4 at the rate of 2 or 4 lb per 40 gallons of spray mixture and the cobalt chloride was mixed at the rate of 1 lb/40 gallons. Within one week of application, there was an obvious difference in foliar response to the herbicide. Triclopyr alone, either at the 2 or 4 lb rate of application, resulted in only a partial kill whereas with the combination of triclopyr plus cobalt chloride all leaves were dead (Fig. 17). Evaluations the following summer in 1991 revealed that with triclopyr alone at both rates of application regrowth occurred but in the presence of the cobalt chloride there

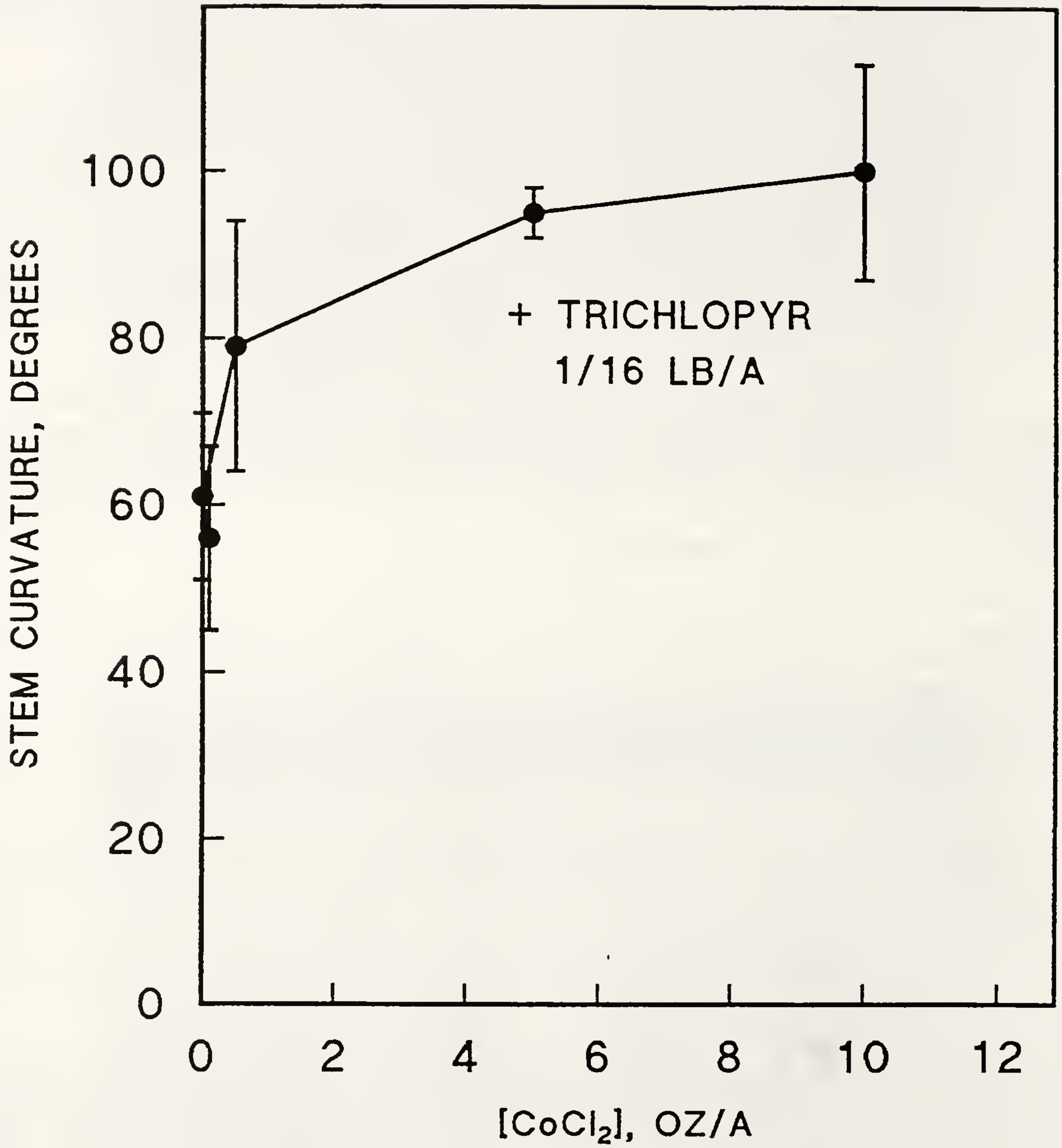


Figure 13. Curvature of garden bean in response to 1/16 lb/A triclopyr (Garlon) as a measure of herbicidal response to increasing amounts of cobalt chloride ($CoCl_2$). Plants grown in 6 inch diameter pots in the greenhouse were sprayed as the first trifoliate leaf was beginning to expand. Curvature was measured 2 days after treatment. The response to 5 and 10 oz/A of cobalt chloride was highly significant.

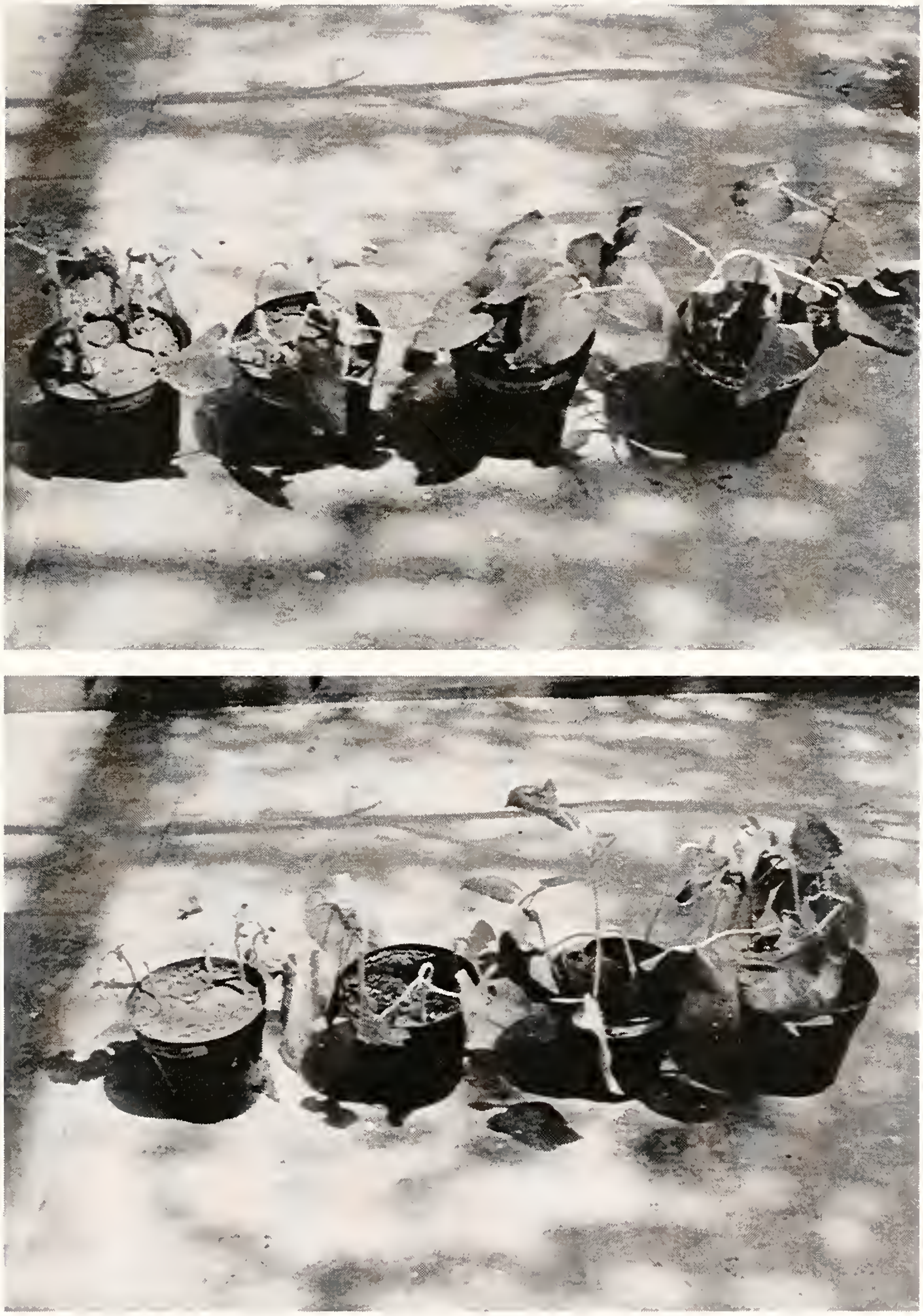


Figure 14. Replicate treatments comparing 1/16 lb/A of triclopyr with (from left to right) decreasing amounts of cobalt chloride of 5 oz/A, 1 oz/A, 0.2 oz/A and 0.02 oz/A. The latter dose was without effect and equal to triclopyr alone. Photographed about 6 days after spraying.

Table 5. Response of garden bean in the greenhouse to 1/4 oz/A of chlorsulfuron (Telar) in combination with 1/16 lb/A of triclopyr (Garlon) with and without cobalt chloride. Results are averages of 5 (plus cobalt) or 7 (minus cobalt) replications plus or minus standard deviations. Evaluations were 2 to 3 days post treatment.

Garlon	Chlorsulfuron	Cobalt chloride	Curvature, degrees
None	None	None	0 ± 0^a
None	1/4 oz/A	None	0 ± 0^a
None	None	10 oz/A	0 ± 0^a
None	1/4 oz/A	10 oz/A	0 ± 0^a
1/16 lb/A	None	None	95 ± 13^b
1/16 lb/A	1/4 oz/A	None	96 ± 9^b
1/16 lb/A	None	10 oz/A	113 ± 9^c
1/16 lb/A	1/4 oz/A	10 oz/A	136 ± 8^d

Numbers not followed by the same letter are significantly different ($p > 0.05$).

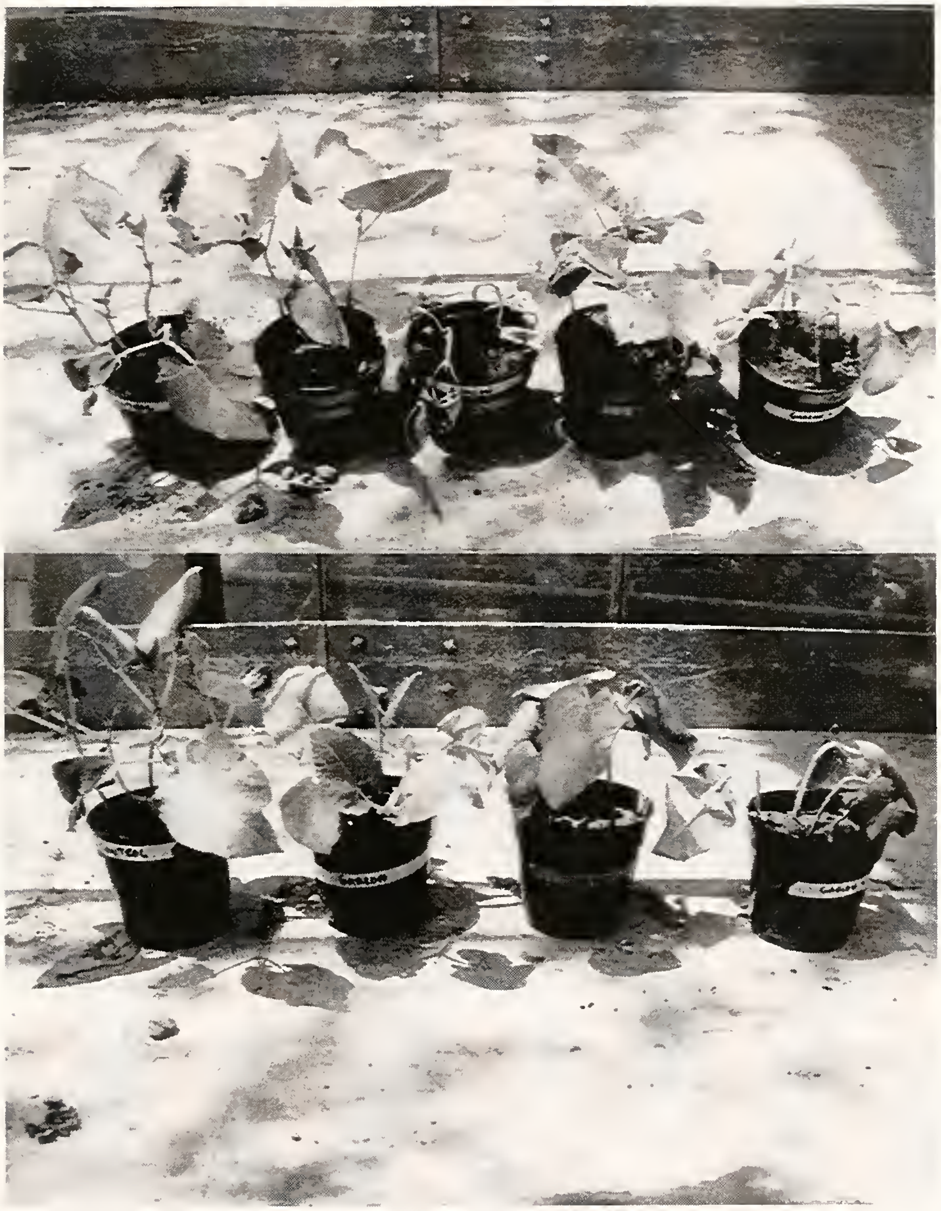


Figure 15. Garden bean treated with 1/16 lb/A triclopyr (Garlon) or chlorsulfuron (Telar) with (upper row) or without (lower row) 10 oz/A of cobalt chloride. A. From let to right. Control cobalt chloride alone, triclopyr plus cobalt chloride, chlorsulfuron plus cobalt chloride and triclopyr plus chlorsulfuron plus cobalt chloride. B. Control, triclopyr, chlorsulfuron, triclopyr plus chlorsulfuron. Photographed 4 days after treatment.

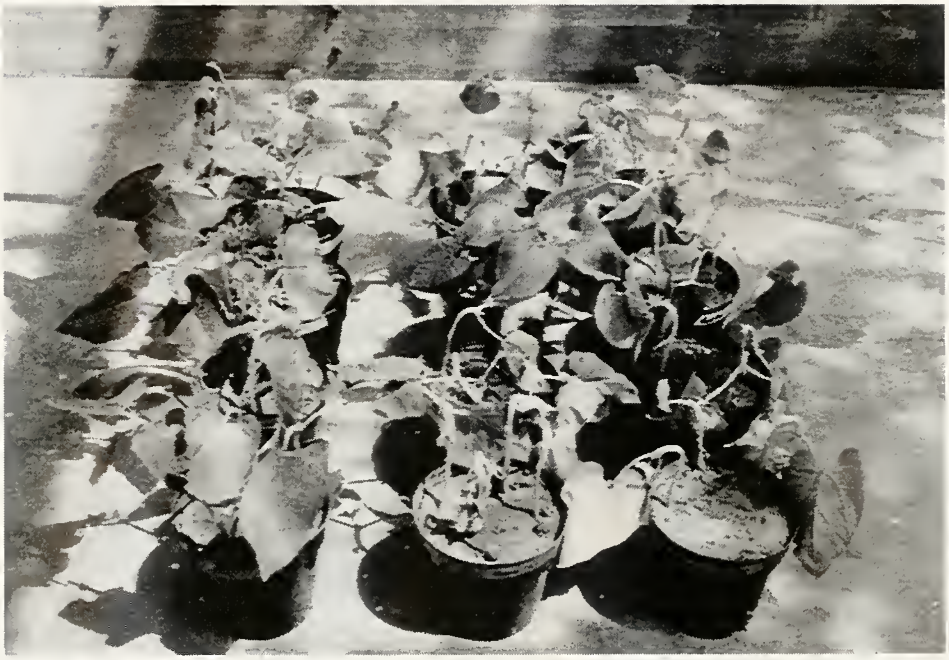


Figure 16. Bean plants treated with increasing amounts of cobalt chloride in combination with triclopyr (Garlon) or triclopyr plus chlorsulfuron (Telar). Back row: 0.01 oz/A cobalt chloride. Second row: 0.1 oz/A cobalt chloride. third row: 0.5 oz/A cobalt chloride. Front row: 5 oz/A cobalt chloride. Treatments were (from left to right); none, 1/16 lb/A triclopyr, and 1/16 lb/A triclopyr plus 1/4 oz/A chlorsulfuron. Plants were photographed approximately 6 days after spraying. Greenhouse test.

Table 6. Response of garden bean plants in the greenhouse to triclopyr (Garlon) and triclopyr plus chlorsulfuron (Telar) comparing cobalt chloride and ammonium nitrate additives.

Triclopyr	Chlorsulfuron	Cobalt chloride	Ammonium nitrate	Degrees of Stem curvature
None	None	None	None	0 ± 0^{a}
None	None	5 oz/A	None	0 ± 0^{a}
None	None	None	5 oz/A	0 ± 0^{a}
None	None	None	20 oz/A	0 ± 0^{a}
1/16 lb/A	None	None	None	67 ± 15^{b}
1/16 lb/A	None	5 oz/A	None	95 ± 5^{c}
1/16 lb/A	None	None	5 oz/A	104 ± 5^{c}
1/16 lb/A	None	None	20 oz/A	117 ± 4^{d}
1/16 lb/A		None	None	100 ± 8^{c}
1/16 lb/A		5 oz/A	None	$114 \pm 4^{c,d}$
1/16 lb/A		None	5 oz/A	$92 \pm 7^{b,c}$
1/16 lb/A		None	20 oz/A	125 ± 5^{e}

Results are averages of two groups of three treated plants each ± mean average deviation. Means not followed by the same letter are significantly different ($p < 0.05$).

was no regrowth.

In the second evaluation applied on September 5, 1990, the rate of Garlon-4 (triclopyr) was fixed at 2 lb per 40 gallons, Telar (chlorsulfuron) was included at the rate of 1/4 oz per 100 gallons and cobalt chloride and ammonium nitrate were compared at 1 lb per 40 gallons. The test site was located on South River Road in Tippecanoe County. Species were again elm, mulberry and poison ivy. There was complete control of all species treated and the cobalt chloride and ammonium nitrate were equivalent.

The third and final evaluation in 1990 was applied September 15, 1990 to a fence row also located on South River Road in Tippecanoe County. Compared were triclopyr (Garlon-4) alone at 2 lb/40 gal in combination with either cobalt chloride or ammonium nitrate at the rate of 1 lb/A. Species treated were elm, hackberry, mulberry and honeysuckle. Evaluations the following spring indicated virtually complete control with no regrowth and that the cobalt chloride and ammonium nitrate additives were approximately equal in their effectiveness.

In 1991, the mixtures of triclopyr and cobalt chloride and of triclopyr and ammonium nitrate were more extensively tested according to the schedules and locations given in Table 7. Applications were with truck-mounted Swinglok equipment operated by Mr. Dave Sosbe under the direction of Mr. Don Bickel of the Crawfordsville Subdistrict INDOT. The treated areas were evaluated at monthly intervals following spraying in 1991 and again in the spring and summer of 1992 to evaluate regrowth.

In general, both the mixture of triclopyr with cobalt chloride or the mixture of triclopyr with ammonium nitrate performed well (Figs 18 and 19). The results were judged to have been equivalent or even superior to what might have been obtained under similar conditions using previously recommended mixtures based on 2,4,5-T or Sislvex. For most species the sprayed foliage was killed completely within one month of application and for those plants or plant parts where the foliage was killed, there was no subsequent regrowth the following season. The mixture could be used either to kill brush by overspraying to stop encroachment (Fig. 19) or to chemically prune to improve sight distances on road segments lined by larger trees and brush (Fig. 18). The utility of the treatment in chemical pruning was especially well demonstrated in the trials applied on SR-47 (Table 7 continued, last half) in 1991. The unsprayed portions, especially of larger trees, seem not to be adversely affected by the treatment, yet the sprayed branches and limbs die and fall away.

The species controlled by the mixture are summarized in Table 8. Very sensitive species include briars (raspberry and blackberry), mulberry, walnut, sumac, sassafras, box elder, buckeye, cottonwood, wild grape and poison ivy. Sensitive species include red bud, elm, honey locust, hackberry, black locust, willow, sycamore, osage orange, multiflora rose, alternate-leaved dogwood and white oak. Black cherry, hard maple and honeysuckle were intermediate. Resistant species included green briar, bittersweet, juniper and ash. There was no injury to grass.

As a result of the 1991 field trials, it appeared that the very sensitive and sensitive species all were killed at all rates of application down to and

Table 7. 1991 field trials of triclopyr plus cation additive mixtures for control of roadside brush.

Cobalt chloride mixture

260 gallons of water

6.6 lb cobalt chloride

5 gallons Garlon 3 A

Brush was sprayed on I-65 south of Wabash River. Also sprayed was SR-25 from I-65 to Delphi and back to 1.5 miles south of Tippecanoe/Carroll County line.

Ammonium nitrate mixture

130 gallons of water

3.3 lb ammonium nitrate

2.5 gallons Garlon 3 A

Brush was sprayed from 1.5 miles south of the Tippecanoe/Carroll County line to Wildcat Creek on SR-25 south of I-65. Also sprayed was the curve on I-231 and Teal Road intersection southbound and north of Linden overpass sprayed southbound.

Applications were on July 17, 1991. Evaluations were on August 29, August 30 and September 2, 1991 and the following summer in 1992.

The above mixtures were also applied at 1/2 strength to the following locations:

Cobalt chloride mixture (half strength). Applied August 12, 1991

I-74, starting at bridge west of US 41 eastbound to I-465 and back to bridge by golf course.

Ammonium nitrate mixture (half strength). Applied August 13, 1991

I-74, starting at golf course and continuing westbound until load emptied approximately at intersection with US-231.

Table 7 (Continued) 1991 field trials of triclopyr plus cation additive mixtures for control of roadside brush.

Mixtures containing reduced amounts of cobalt chloride or ammonium nitrate

I-74E from Jct 41 to I-465 and back to Eagle Creek

390 gallons water

4.4 lb. cobalt chloride

7.5 gallons Garlon 3A

I-74W from Eagle Creek to SR-25

200 gallons water

2.2 lb. ammonium nitrate

3.5 gallons Garlon 3A

Mixtures containing both reduced amounts of cation additives and of triclopyr (Garlon)

SR-47S from SR-32 to Lake Waveland and back to Brownsburg in the Crawfordsville district

260 gallons water

2.2 lb cobalt chloride

2.5 gallons Garlon 3A

SR-47N from Brown's Valley to SR-32 and US-231 from SR-234 to Mall entrance

130 gallons water

1.1 lb ammonium nitrate

1.25 gallons Garlon 3A

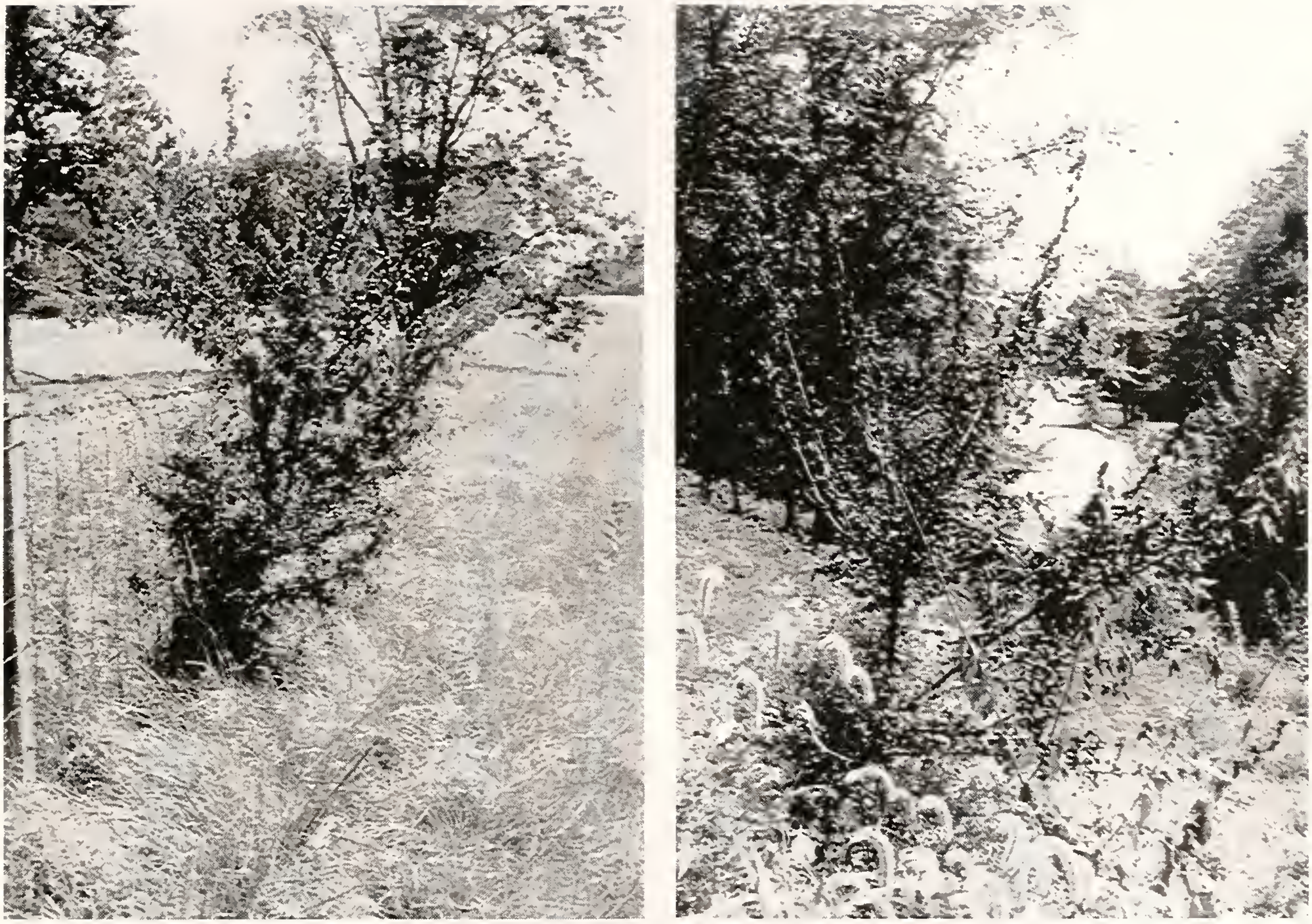


Figure 17. Results of the initial evaluation of triclopyr plus the cobalt chloride additive on roadside brush. Treatments were on August 27, 1990. The trees were photographed on September 1, 1990.

A. A mulberry treated with 2 lb/40 gallons of water of triclopyr (Garlon) alone. The foliage was less than 50% killed and the tree survived.

B. A mulberry treated with 2 lb/ 40 gallons of water of triclopyr (Garlon) plus 1 lb/40 gallons water of cobalt chloride. All leaves were dead and the tree did not survive.

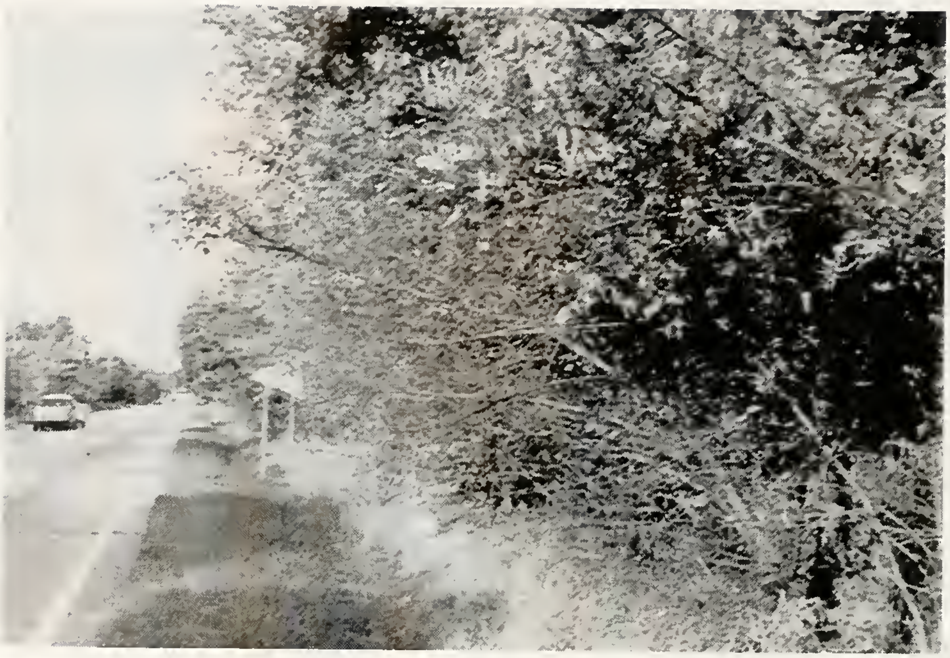


Figure 18. Portion of SR-25N between Lafayette and Delphi treated with the mixture of triclopyr (Garlon) plus cobalt chloride given in Table 7. The application was on July 17, 1991. The photograph was taken September 2, 1991. Note the effectiveness in chemical pruning.

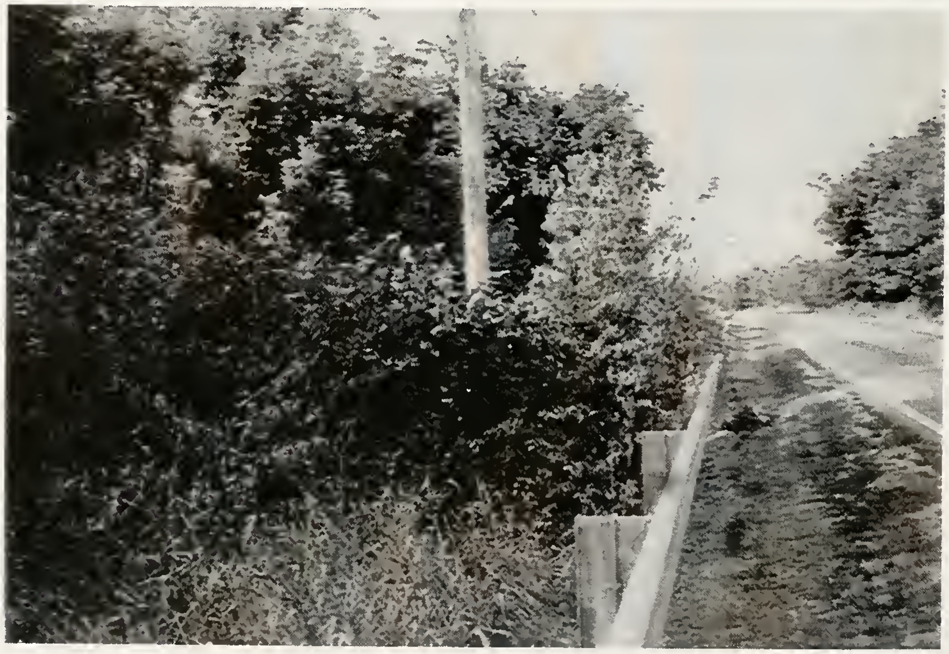


Figure 19. Portion of SR-25S between Delphi and Lafayette treated with the mixture of triclopyr (Garlon) and ammonium nitrate given in Table 7. The application was on July 17, 1991. The photograph was taken September 2, 1991. The treatment was nearly equivalent to that with cobalt chloride of Figure 18.

Table 8. Species controlled

Very sensitive	Sensitive	Intermediate	Resistant
Briars (blackberry, raspberry)	Red Bud	Hard maple	Juniper
Mulberry	Elm	Black cherry	Ash
Walnut	Honey locust		
Sumac	Black locust		
Sassafras	Hackberry		
Box elder	Willow		
Buckeye	White oak		
Cottonwood			
Wild grape			
Poison ivy			

including a 1/4 strength mixture. The mixture applied at half strength was still effective but the mixture at 1/4 strength appears perhaps too light for best results. It was still acceptable for the cobalt additive but not for the ammonium nitrate additive. Control of hard maple was as good or better at the 1/4 strength rate but control of elm and mulberry was spotty.

Evaluations of regrowth made in late June of 1992 confirmed the 1991 evaluations. Ash and maple, the two resistant species, were 50% and 10 to 20% controlled respectively. Dead portions of the trees did not regrow but only about half to three-fourths of the sprayed hard maple and ash was actually killed. Honey locust was 80% controlled and box elder was 80 to 90% controlled. All other susceptible or very susceptible species listed in Table 8 were killed and did not regrow.

The 1990 and 1991 trials as well as additional trials applied in 1992 (Table 9) demonstrated the general effectiveness of the triclopyr (Garlon)-cation (cobalt chloride or ammonium nitrate) mixture for general control of roadside brush. As a result of the implementation trials of 1991 and 1992, according to Dr. Don Bickel of the Crawfordsville Subdistrict, the Crawfordsville Subdistrict no longer has roadside brush problems and future implementation activities must, by necessity, be extended to other subdistricts.

3. Modifications of the brush control mixture to achieve control of the resistant species ash and hard maple

The mixture of triclopyr (Garlon) plus cation (cobalt chloride or ammonium nitrate) was very effective in the control of roadside brush except for two species, ash and hard maple, which were only partially controlled even at the highest rates of application. Therefore, a series of trials were initiated beginning in 1991 to attempt to modify the mixture to provide for control of the resistant species as well.

a) Field trials with dicamba (Banvel).

A survey of commercially available materials suggested that dicamba (Banvel) should have good activity against ash. Therefore, it was among the first of a new series of additives tested in the brush mixture to improve activity against resistant species. In the first trial, applied on September 9, 1991, 0.5 lb/A of dicamba (Banvel) was included in a mixture of 2 lb of triclopyr and 1 lb of cobalt chloride (all per 40 gallons of water. Ash trees, between 10 and 20 feet tall were treated. The dicamba additive was compared to the same mixture lacking dicamba. Also treated were hard maple trees between 3 and 15 feet in height. The addition of dicamba to the mixture may have improved the activity against ash and hard maple somewhat but evaluations in the second quarter of 1992 revealed that both ash and hard maple had still only been about 50% controlled by the treatments. Thus the addition of dicamba did not improve treatment effectiveness sufficiently to warrant the additional cost. The addition of dicamba to the brush control mixture was not evaluated further.

b) Field trials with sulfometuron (Oust)

Laboratory and greenhouse studies (Part 6 of this Report) suggested that

Table 9. 1992 filed trials of triclopyr plus cation additive mixtures for control of roadside brush in the Crawfordsville district.

SR-43, starting at Putnam/Montgomery County line south of Parkersburg to SR-67. SR-246 from SR-46 to Clay City. Both sides were sprayed with the cobalt chloride mixture of Table 7.

SR-63 south, starting at SR-32 to junction US-41 at Terre Haute and back north to just south of US-36 with the ammonium nitrate mixture of Table 7.

Additional areas sprayed include:

SR-32 east of Crawfordsville

US-36 west of Montezuma

SR-43 north of West Lafayette

Applications were in mid-July with truck-mounted equipment under the direction of Mr. Don Bickel.

chlorsulfuron (Telar), a sulfonylurea herbicide, might, under certain conditions, augment the action of the combination of triclopyr and cobalt chloride or ammonium nitrate. Therefore, a series of field trials were set up in 1992 to evaluate this possibility. Upon consultation with scientists at DuPont, the sulfonylurea chosen for field testing was sulfometuron (Oust) rather than chlorsulfuron (Telar). However, at some point chlorsulfuron still should be field tested.

All tests with sulfometuron were under field conditions at a rate in the mixture of 0.1 lb per 40 gallons of spray mixture. Treatments were restricted to ash and hard maple, the two species resistant to the triclopyr/cation mixtures. Surfactant also was included in an attempt to increase treatment effectiveness (Tables 10-14).

In comparisons with triclopyr and cobalt chloride (Tables 11, 12 and 14), the addition of 0.1 lb/40 gallons of spray mixture of sulfometuron (Oust) was without effect or antagonistic. In comparisons with triclopyr and ammonium nitrate (Tables 10 and 13), there was an effect of sulfometuron with ash and only a small and insignificant improvement with hard maple.

4. Development of a new series of additives to enhance the effectiveness of the triclopyr-cation-surfactant brush control mixture in the control of ash and hard maple.

a) Introduction

In an effort to solve the problem with regard to the lack of effectiveness of the triclopyr-cation-surfactant mixtures on resistant species, ash and hard maple (Tables 10-14), a program was initiated to reinvestigate the first part of the inhibitory scheme of Figure 2. The objective was to discover and develop an additive more effective than chlorsulfuron (or sulfmeturon) in modulating this part of the pathway. A large body of basic studies were carried out and completed and a summary of these studies is available as Appendix I to this report.

b) Laboratory studies

The basic laboratory approach was to investigate the auxin herbicide-stimulated NADH oxidase of isolated plasma membrane vesicles of soybean using the same experimental protocols as described in Part 2 of this report. Some characteristic, other than inhibition by chlorsulfuron, was sought that could be exploited in the development of a new and more-effective additive.

Basically, the findings relevant to the new and more-effective additive discovered and developed are summarized as follows: Details are summarized as Appendix I of this report.

i) The auxin herbicide-stimulated NADH oxidase activity of plasma membrane vesicles from stems of dark-grown seedlings of soybean was found to have one or more thiol groups (-SH) at or near the substrate- or auxin-binding sites.

ii) A group of substances known collectively as thiol reagents were found

Table 10. Comparison of triclopyr (Garlon 4) and ammonium nitrate additive combined with sulfometuron (DPX-5648 = Oust) on control of ash and hard maple. Treatments applied July 28, 1992. IN-126 test area. Evaluated August 28, 1992.

Amount/40 gallons water				% Defoliation	
Triclopyr	Ammonium nitrate	X-77 Surfactant	Sulfometuron	Ash	Hard maple
2 lb	1 lb	0.1 gal*	--	53 ± 35	46 ± 27
2 lb	1 lb	0.1 gal	0.1 lb	58 ± 30	80 ± 10

*0.25% of the total spray mixture

Table 11. Comparison of triclopyr (Garlon 4) and cobalt chloride additive combined with sulfometuron (DPX-5648 = Oust) on control of ash and hard maple. Treatments applied September 6, 1992. IN-126 test area. Evaluated September 19, 1992 and October 18, 1992.

Amount/40 gallons water				% Defoliation			
				Ash		Hard maple	
Triclopyr	Cobalt chloride	X-77 Surfactant	Sulfometuron	Sept 19	Oct 18	Sept 19	Oct 18
2 lb	1 lb	0.1 gal*	--	20 ± 10	60 ± 10	20 ± 10	90 ± 10
2 lb	1 lb	0.1 gal	0.1 lb	40 ± 10	35 ± 5	0 ± 0	75 ± 5

*0.025% of the total spray mixture

Table 12. Comparison of triclopyr (Garlon 4) and cobalt chloride additive combined with sulfometuron (DPX-5648 = Oust) on control of ash and hard maple. Treatments applied September 14, 1992. IN-126 test area. Evaluated September 19, 1992 and October 18, 1992.

Amount/40 gallons water				% Defoliation			
				Ash		Hard maple	
Triclopyr	Cobalt chloride	X-77 Surfactant	Sulfometuron	Sept 19	Oct 18	Sept 19	Oct 18
2 lb	1 lb	0.1 gal*	--	30 ± 10	80 ± 10	10 ± 10	90 ± 10
2 lb	1 lb	0.1 gal	0.1 lb	30 ± 10	55 ± 5	10 ± 10	75 ± 25

*0.025% of the total spray mixture

Table 13. Comparison of triclopyr (Garlon 4) and ammonium nitrate additive combined with sulfometuron (DPX-5648 = Oust) on control of ash and hard maple. Treatments applied July 28, 1992. IN-126 test area. Evaluations were on October 10 and October 18, 1992.

	Amount/40 gallons water			% Defoliation	
Triclopyr	Ammonium nitrate	X-77 Surfactant	Sulfometuron	Oct 10	Oct 18
2 lb	1 lb	0.1 gal*	--	15 ± 5	40 ± 10
2 lb	1 lb	0.1 gal	0.1 lb	15 ± 30	50 ± 40

*0.25% of the total spray mixture

Table 14. Comparison of triclopyr (Garlon 4) and ammonium nitrate additive combined with sulfometuron (DPX-5648 = Oust) on control of ash. Treatments applied October 2, 1992. Evaluations were on October 8, 10, 18 and 25, 1992. 1992. IN-126 test area.

	Amount/40 gallons water			% Defoliation		
Triclopyr	Choline chloride	X-77 Surfactant	Sulfometuron	Oct 10	Oct 18	Oct 25
2 lb	1 lb	0.1 gal*	--	0 ± 0	50 ± 10	70 ± 10

*0.25% of the total spray mixture

to both stimulate and inhibit specifically auxin herbicide-induced growth depending on concentration. Basal (auxin-independent) or control growth was largely unaffected by thiol reagents.

iii) These same thiol reagents both stimulated or inhibited specifically the auxin herbicide-induced component of the NADH oxidase. The basal oxidase activity was unaffected by the thiol reagents.

iv) The auxin-herbicide reactive thiol could be marked in the membrane if all available thiols in the presence of 2,4-D were reacted with a thiol alkylating reagent such as N-ethylmaleimide, after which the 2,4-D was removed and any thiol groups protected by the presence of 2,4-D could then be located by a second thiol alkylating reagent combined with a reporter molecule. In this manner, two proteins of relative molecular weights of 36,000 and 72,000 were identified as components of the auxin herbicide-responsive NADH oxidase complex.

v) Based on the studies of Appendix I summarized above, three compounds were selected for further greenhouse and field evaluations. These compounds are listed in Table 15 and for purposes of this report were designated TR-I, TR-II and TR-III.

To further evaluate the additives TR-I and TR-II in the laboratory, cutting of woody plants were propagated in tissue culture. Micro cuttings of apple (*Malus domestica*), *Dipladenia* and *Daphne ordorata*, the latter both ornamental shrubs, were grown in agar-containing growth factors and a mineral mixture. Several criteria were evaluated including root formation, root growth, cutting growth and callus formation. With both TR-I and TR-II the action of indole-3-acetic acid as an auxin was enhanced according to a definite optimum curve. For all three species and both additives, the optimum was achieved in the dose range 0.1 to 1 mM with either higher or lower concentrations being less effective. Based on these findings, and on the laboratory studies with the growth of soybean sections, it was predicted that the TR series of compounds might be active over a relatively narrow range of concentrations. Therefore, it appeared important to determine dose-response relationships for the TR additives both in the greenhouse and in the field (see below).

c) Greenhouse studies

The thiol reagent additives, TR-I and TR-II, identified from laboratory studies above, were evaluated further in the greenhouse for their ability to enhance the herbicidal activity of triclopyr on plants of garden bean (Table 16). Neither TR-I nor TR-II exhibited herbicidal activity alone or in the presence of cobalt chloride. However, in combination with triclopyr plus the cobalt chloride additive, both TR-I and TR-II exerted a synergistic effect with triclopyr that resulted in more rapid plant killing and a reduced fresh weight approximately one week following treatment.

Based on these results a third TR additive, TR-III, also was greenhouse tested. It, too, was without effect by itself but resulted in a synergistic herbicidal interaction with triclopyr (Table 17). The synergistic interaction was seen as an enhanced degree of stem curvature (Table 17, Fig. 20) and a decreased plant height (Table 18, Fig. 20). Fresh weight per plant also was

Table 15. Thiol reagents selected from laboratory studies for greenhouse and field evaluations as brush control additives.

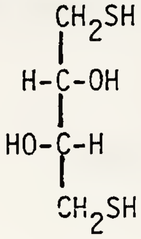
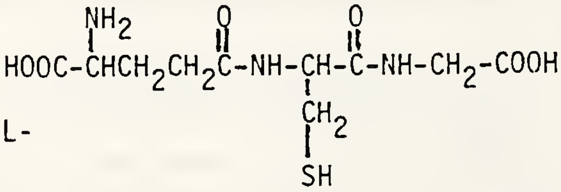
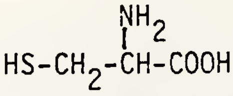
Designation	Chemical name	Chemical structure
TR-I	Dithiothreitol Abbreviated: DTT (threo-1,4-dimercapto-2,3-butanediol = Cleland's reagent) FW 154.25	 $HSCH_2-CH(OH)-CH(OH)-CH_2SH$
TR-II	Glutathione, reduced Abbreviated: GSH (Y-1-GLUTAMYL-1-CYSTEINYL-glycine) FW 307.33	 $HOOC-CH(NH_2)CH_2CH_2C(=O)-NH-CH(CH_2SH)-C(=O)-NH-CH_2-COOH$
TR-III	DL-Cysteine Abbreviated: CYS FW 121.16	 $HS-CH_2-CH(NH_2)-COOH$

Table 16. Effect of TR-I and TR-II on the herbicidal activity of triclopyr plus cobalt chloride and surfactant on garden bean plants in the greenhouse.

Amount per 40 gal water					
Triclopyr	Cobalt chloride	TR-I	TR-II	Plants dead, %	Weight /plant, grams
None	None	None	None	0 ± 0^{a}	0.95^{a}
None	10 oz/A	None	None	0 ± 0^{a}	0.98 ± 0.16^{a}
None	10 oz/A	1 lb/A	None	0 ± 0^{a}	0.91 ± 0.17^{a}
None	10 oz/A	None	1 lb/A	0 ± 0^{a}	1.17 ± 0.08^{a}
1/16 lb/A	None	None	None	71 ± 6^{b}	0.55 ± 0.2^{b,c}
1 16 lb/A	10 oz/A	None	None	54 ± 12^{b}	0.45 ± 0.05^{b}
1/16 lb/A	10 oz/A	1 lb/A	None	63 ± 37^{b}	0.30 ± 0.2^{b,c}
1/16 lb/A	10 oz/A	None	1 lb/A	100 ± 0^{c}	0.35 ± 0.05^{c}

Values not followed by the same letter are statistically significant ($p < 0.05$)

Table 17. Herbicidal response of triclopyr (Garlon) plus cobalt chloride and surfactant enhanced by additive TR-III (Table 15). Results are averages from two pots with 3 or 4 plants each ± mean average deviations. Evaluations were made 48 hours after spraying.

Triclopyr	Cobalt chloride	Additive/Rate	Stem curvature, degrees
None	None	None	0 ± 0[a]
1/16 lb/A	1 lb/A	None	21 ± 8[b]
1/16 lb/A	1 lb/A	TR-III, 1 lb/A	57 ± 14[c]
1/16 lb/A	1 lb/A	N-chlorosuccinamide, 1 lb/A	16 ± 5[b]
None	None	TR-III, 1 lb/A	0 ± 0[a]
None	None	N-chlorosuccinamide, 1 lb/A	0 ± 0[a]

X-77 surfactant was included at a rate of 0.25% of the total spray mixture. Values not followed by the same letter are significantly different ($p < 0.05$)

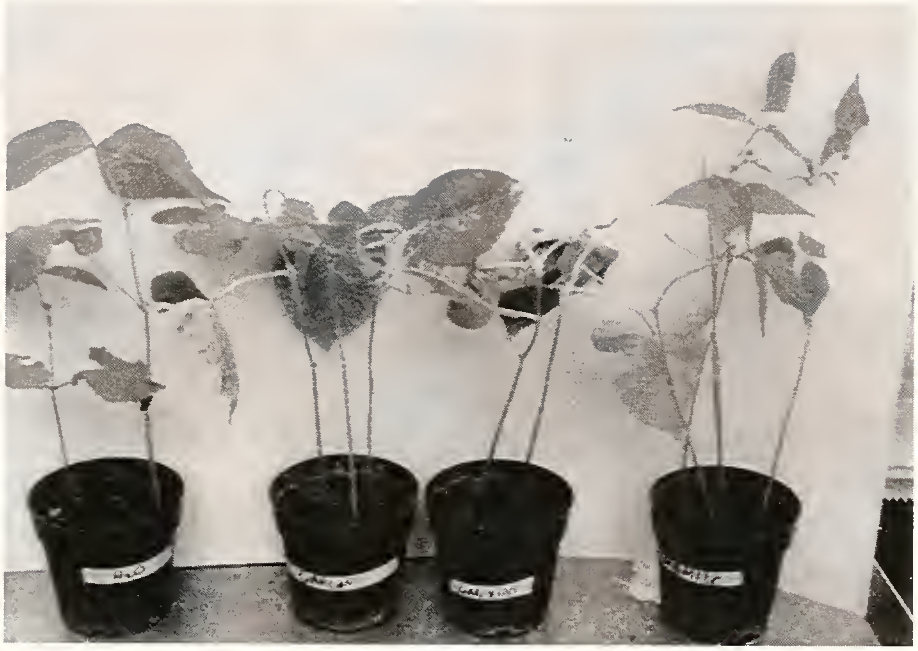


Figure 20. Garden bean 48 h after treatment. From left to right: No treatment, triclopyr (Garlon-4) 1/16 lb/A, triclopyr 1/16 lb/A plus TR-III (Cysteine) 1 lb/A, triclopyr 1/16 lb/A plus N-chlorosuccinamide 1 lb/A. Herbicidal activity of the triclopyr was greatly enhanced by the TR-III additive and an antagonistic response was observed with N-chlorosuccinamide.

Table 18. Herbicidal response of triclopyr (Garlon) enhanced by the additive TR-III (Table 15). Results are averages from two pots with 3 or 4 plants each ± mean average deviations. Plants were treated about 1 week after planting. Evaluations were 12 days after treatment. Plants were grown and observed in the greenhouse. Each pot contained 3 to 5 plants.

Triclopyr	Additive	Height, inches	Fresh weight, grams/plant
None	None	12.0 ± 1.0^{a}	5.3 ± 2.0
None	TR-III, 2.75 lb/A	12.3 ± 0.7^{a}	-
None	N-chlorosuccinamide, 2.75 lb/A	12.8 ± 0.6^{a}	5.2 ± 2.5
0.2 lb/A	None	10.5 ± 0.5^{b}	4.2 ± 1.0
0.2 lb/A	TR-III, 2.75 lb/A	7.8 ± 0.8^{c}	3.3 ± 0.5
0.2 lb/A	N-chlorosuccinamide, 2.75 lb/A	$11.0 \pm 1.0^{a,b}$	4.0 ± 1.7

decreased by TR-III but the decrease was not statistically significant (Table 18). N-chlorosuccinamide, a weak oxidizing agent, having a mode of action exactly opposite to that of TR-I, -II and -III, was antagonistic to triclopyr (Fig. 20) or without effect (Tables 17 and 18).

Preparatory to field trials, a dose-response relationship was determined for TR-III in combination with triclopyr in the greenhouse (Figs. 21 and 22). At 0.2 lb/A triclopyr, a bimodal synergism with TR-III was observed with response maxima at 0.01 and 0.1 lb/A in terms of plant height (Fig. 21), stem curvature response (Fig. 22A) and fresh weight per plant (Fig. 22B).

d) Field studies

Beginning in the fall of 1992, the TR additives were field tested primarily on ash and hard maple, the two species most resistant to the triclopyr (Garlon-4)-cobalt chloride/ammonium nitrate herbicide mixture developed in the first half of the project period. In these studies, the rate of triclopyr was fixed at 2 lb active material per 40 gallons of spray mixture and the rate of cobalt chloride or ammonium nitrate was fixed at 1 lb/A. In addition 0.1 gal or 0.25% of the total spray volume of X-77 surfactant was included.

In the first trials, TR-I (DTT) did enhance the activity of triclopyr plus cobalt chloride on ash (Table 19). In a subsequent comparison, activity was enhanced on ash and hard maple for TR-I but not for TR-II (GSH) (Table 20). In fact, TR-II may have been antagonistic in its action at the rate tested. TR-I was beneficial, as well in the combination of triclopyr plus ammonium nitrate both in the presence and absence of Sulfometuron (Oust) (Table 21).

As a specificity control, the cobalt chloride or ammonium nitrate of the mixtures was replaced in two experiments by magnesium chloride. The magnesium is a cation predicted to have effects opposite to that of cobalt or ammonium. This prediction was borne out in that in both experiments where triclopyr was added in the presence of magnesium, it was less effective than in the absence of magnesium (Table 22). The herbicidal action was slowed by the magnesium and overall effectiveness was reduced. However, even in the presence of magnesium, TR-II enhanced the herbicidal activity of the triclopyr in the defoliation of hard maple (Table 22).

In Table 23, data for TR-III (Cysteine) are included as well and compared to TR-I and TR-II in the triclopyr plus cobalt chloride combination on ash. Enhanced activity was seen for both TR-I and TR-III but not for TR-II (Table 23). When tested in combination with triclopyr, cobalt chloride and sulfometuron (Table 24), both TR-I and TR-III enhanced significantly the effectiveness of the mixture by 3 weeks after the treatments were applied. Again, TR-II was less effective than the three-way mixture of triclopyr, cobalt chloride, surfactant and sulfometuron alone.

As a final field evaluation, TR-III was added to the mixture of triclopyr plus ammonium nitrate and surfactant. Significant enhancement was observed at an application rate of 1 lb TR-III per 40 gallons of spray mixture (Table 25).

To set the rate of TR-III, a dose response was carried out in the field

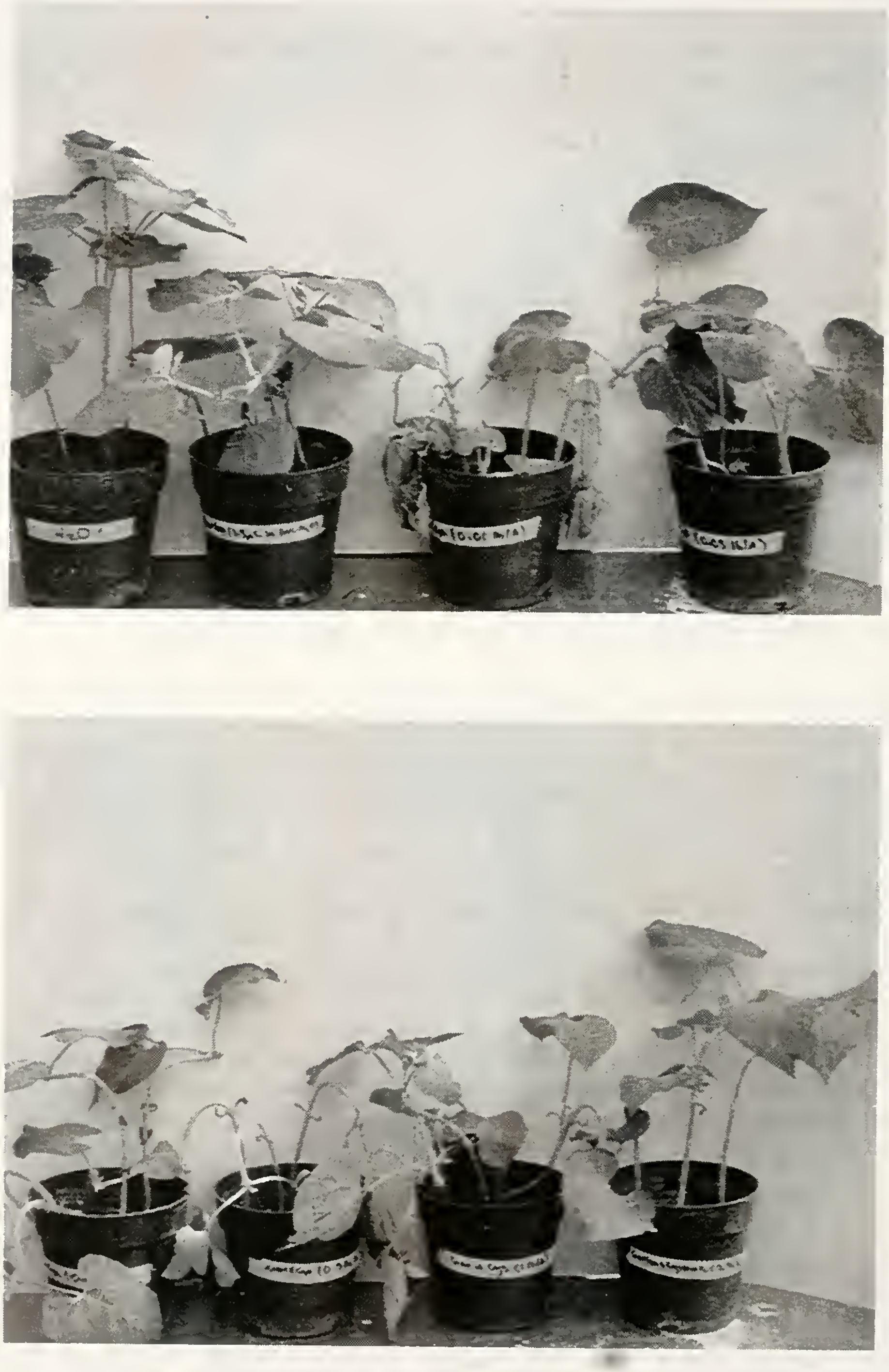


Figure 21. Dose response of garden bean treated with no triclopyr or triclopyr at 0.2 lb/A plus varying concentrations of additive TR-III (Cysteine). Photographed 72 h after treatment.

A. Upper photograph. From left to right: No treatment, triclopyr at 0.2 lb/A, triclopyr 0.2 lb/A plus cysteine 0.01 lb/A, triclopyr 0.2 lb/A plus cysteine 0.05 lb/A.

B. Lower photograph. From left to right: triclopyr 0.2 lb/A plus cysteine 0.1 lb/A, triclopyr 0.2 lb/A plus cysteine 0.5 lb/A, triclopyr 0.2 lb/A plus cysteine 1 lb/A, triclopyr 0.2 lb.A plus cysteine 2 lb/A.

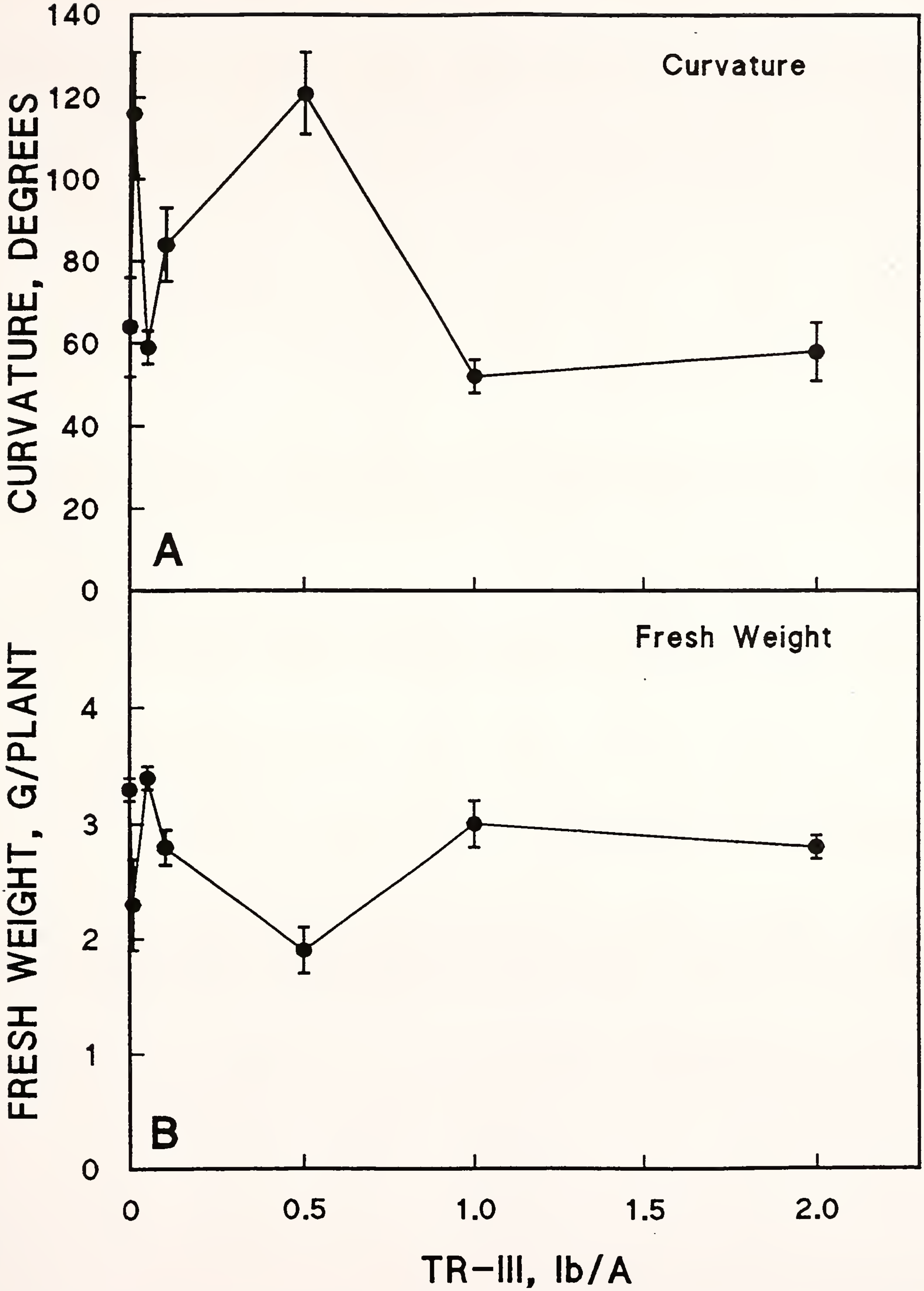


Figure 22. Response of garden bean treated in the presence of 0.2 lb/A of triclopyr (Garlon-4) to increasing concentrations of TR-III (Cysteine) additive. A. Upper curve = stem curvature measured 24 h after treatment. B. = Fresh weight per plant measured 7 days after treatment. Beans were sprayed 8 days after planting. results are means of two pots of 5 plants each ± mean average deviations.

Table 19. Comparison of triclopyr (Garlon-4) and the cobalt chloride additive combined with TR-I (DTT) on control of ash and hard maple. IN-126 test area. Treatments were applied September 6, 1992. Evaluations were on September 19 and October 18, 1992.

Amount/40 gallons water				% Defoliation			
				Ash		Hard maple	
Triclopyr	Cobalt chloride	X-77 Surfactant	TR-I	Sept 19	Oct 18	Sept 19	Oct 18
2 lb	1 lb	0.1 gal*	None	0	35 ± 5	40	60 ± 10
2 lb	1 lb	0.1 gal	1 lb	10 ± 10	80	10	50

*0.25% of the total spray mixture

Table 20. Comparison of triclopyr (Garlon-4) and the cobalt chloride additive combined with TR-I (DTT) and TR-II (GSH) on control of ash and hard maple. IN-126 test area. Treatments applied September 12, 1992. Evaluations were on September 19 and October 18, 1992.

Amount/40 gallons water					% Defoliation			
					Ash		Hard maple	
Triclopyr	Cobalt chloride	X-77 Surfactant	TR-I	TR-III	Sept 19	Oct 18	Sept 19	Oct 18
2 lb	1 lb	0.1 gal*	None	None	20	60 ± 10	20	90 ± 10
2 lb	1 lb	0.1 gal	1 lb	None	50	100 ± 0	30	100 ± 0
2 lb	1 lb	0.1 gal	None	1 lb	30	40 ± 10	40	70 ± 10

*0.25% of the total spray mixture

Table 21. Comparison of triclopyr (Garlon-4) and the ammonium nitrate additive combined with TR-I (DTT) or TR-I plus sulfometuron (Oust) on control of ash. IN-126 test area. Treatments were applied on October 2, 1992. Evaluations were on October 10 and October 18, 1992.

Amount/40 gallons water					% Defoliation	
Triclopyr	Cobalt chloride	X-77 Surfactant	TR-I	Sulfometuron	Oct 10	Oct 18
2 lb	1 lb	0.1 gal*	None	None	15 ± 5	40 ± 10
2 lb	1 lb	0.1 gal	1 lb	None	35 ± 10	90 ± 10
2 lb	1 lb	0.1 gal	1 lb	0.1 lb	50 ± 10	90 ± 10

*0.25% of the total spray mixture

Table 22. Comparison of triclopyr (Garlon-4) with magnesium chloride replacing the cobalt chloride additive on control of ash and hard maple. IN-126 test area. Results are from two applications in two different locations, one on August 28, 1992 and another on August 29. Evaluations were at two week intervals. The trees died slowly because of the magnesium. Best results were with TR-I (DTT). Results are from a final evaluation on October 28, 1992.

	Amount/40 gallons water				% Defoliation	
Triclopyr	Magnesium chloride	X-77 Surfactant	TR-I	TR-II	Ash	Hard maple
2 lb	1 lb	0.1 gal*	None	None	10 ± 10	30 ± 10
2 lb	1 lb	0.1 gal	1 lb	None	10 ± 10	60 ± 10
2 lb	1 lb	0.1 gal	None	1 lb	10 ± 10	40 ± 10

*0.25% of the total spray mixture. Alternate-leaved dogwood also was treated and only about 10% killed in all three treatments.

Table 23. Comparison of triclopyr (Garlon-4) and the cobalt chloride additive combined with TR-I (DTT), TR-II (GSH) and TR-III (Cysteine) on control of ash. IN-126 test area. Treatments were applied on October 5, 1992. Evaluations were on October 10, October 18 and October 25, 1992.

	Amount/40 gallons water					% Defoliation		
Triclopyr	Cobalt chloride	X-77 Surfactant	TR-I	TR-II	TR-III	Oct 10	Oct 18	Oct 25
2 lb	1 lb	0.1 gal*	None	None	None	20 ± 10	60 ± 10	90 ± 10
2 lb	1 lb	0.1 gal	1 lb	None	None	15 ± 5	90 ± 20	90 ± 10
2 lb	1 lb	0.1 gal	None	1 lb	None	5 ± 5	45 ± 5	90 ± 10
2 lb	1 lb	0.1 gal	None	None	1 lb	15 ± 5	90 ± 20	95 ± 5

*0.25% of the total spray mixture

Table 24. Comparison of triclopyr (Garlon-4) and an additive mixture of cobalt chloride and sulfometuron (Oust) combined with TR-I (DTT), TR-II (GSH) or TR-III (Cysteine) on control of ash (A) and hard maple (B). IN-126 test area. Treatments were applied on October 12, 1992. Evaluations were on October 2??, 1992. Evaluations were on October 10, October 18 and October 25, 1992.

	Amount/40 gallons water						Ash, Defoliation %		
A. Control of ash									
Triclopyr	Cobalt chloride	X-77 Surfactant	Sulfo-meturon	TR-I	TR-II	TR-III	Oct 10	Oct 18	Oct 25
2 lb	1 lb	0.1 gal*	0.1 lb	None	None	None	0 ± 0	50 ± 10	70 ± 10
2 lb	1 lb	0.1 gal	0.1 lb	1 lb	None	None	0 ± 0	60 ± 30	95 ± 5
2 lb	1 lb	0.1 gal	0.1 lb	None	1 lb	None	10 ± 10	40 ± 0	50 ± 0
2 lb	1 lb	0.1 gal	0.1 lb	None	None	1 lb	15 ± 5	100 ± 0	100 ± 0
B. Control of hard maple							Hard maple, Defoliation %		
2 lb	1 lb	0.1 gal	0.1 lb	None	None	None	No data on maple		
2 lb	1 lb	0.1 gal	0.1 lb	1 lb	None	None	20	30	50
2 lb	1 lb	0.1 gal	0.1 lb	None	1 lb	None	10	30	30
2 lb	1 lb	0.1 gal	0.1 lb	None	None	1 lb	15	50	50

*0.25% of the total spray mixture

Table 25. Comparison of triclopyr (Garlon-4) and the ammonium additive combined with TR-III (Cysteine) on control ash and hard maple. IN-126 test area. Treatments were applied on August 10, 1993. Evaluations were on October 14, 1993.

	Amount/40 gallons water			% Defoliation	
Triclopyr	Ammonium nitrate	X-77 Surfactant	TR-III	Ash	Hard maple
2 lb	1 lb	0.1 gal*	None	60 ± 10	50 ± 10
2 lb	1 lb	0.1 gal	1 lb	90 ± 10	100 ± 0

*0.25% of the total spray mixture

similar to that conducted in the greenhouse (Fig. 23). Foliage kill (defoliation, %) was proportional to the logarithm of the rate of application (Fig. 23). After 1 month, 1 lb/40 gal of spray mixture of TR-III has resulted in substantial defoliation of both ash and hard maple. After 2 months, substantial defoliation of ash and hard maple was observed at the 0.1 lb/40 gal application rate as well. Generally, two months after application, the ash and maple of the plots treated with triclopyr and ammonium nitrate containing 0.1 and 1 lb per 40 gal of spray mixture of TR-III were equivalent and completely defoliated (Fig. 24). Other species in these trials including linden, honeysuckle and elm were completely killed by the mixture at all three rates of additive.

Based on these field trials, TR-III was selected as the TR additive of choice for possible implementation trials in 1993 mixed at a rate of between 0.25 and 0.5 pounds per 100 gallons of spray mixture. The exact cost of TR-III is yet to be determined but if purchased in quantity, the cost is estimated to be no more than about $10 per 100 gallons of spray mixture. A derivative of TR-III, N-acetylcysteine, may be effective at lower rates than is TR-III and, if proven effective, could reduce costs even further.

5. To prioritize within the safety zone what trees and brush are undesirable and in what areas.

To initiate prioritization within the safety zone of what trees and brush are undesirable and in what areas, existing information with respect to actual species dominating the roadsides was evaluated. Brush as a maintenance problem was analyzed according to four major categories:

Category I. Brush (or trees) on normal rights-of-way in areas not mowed full-width. As long as Indiana continues a program of full-width mowing, this appears to be sufficient to control Category I brush as determined by spot checks of all major Interstates and four-lane roads.

Category II. Brush (or trees) in areas where mowing is not possible (bridges, cones, guard rails). Category II brush required periodic management. A mid- to late-season broadcast foliar spray is probably the most convenient form of treatment. Category II brush is the only consensus brush problem as viewed by district managers. The decision as to what and when to treat would be made by district maintenance personnel. At present, decisions are based on need and individual judgment. More specific guidelines may be beneficial.

Category III. Encroachment on secondary roads. Category III brush resulting from encroachment must be removed when it constitutes a safety hazard by reducing sight distances. Broadcast basal dormant spraying with an oil-based dormant spray mixture may be most cost effective where the dead stems can be tolerated. The alternative is mechanical removal.

The key to satisfactory management of Category III brush (or trees) is early identification of areas where encroachment may become a problem and overspray by broadcast treatment before the problem develops. District personnel should be alerted to this need and such spraying scheduled regularly as preventative maintenance. Work is in progress to develop objective criteria for determining

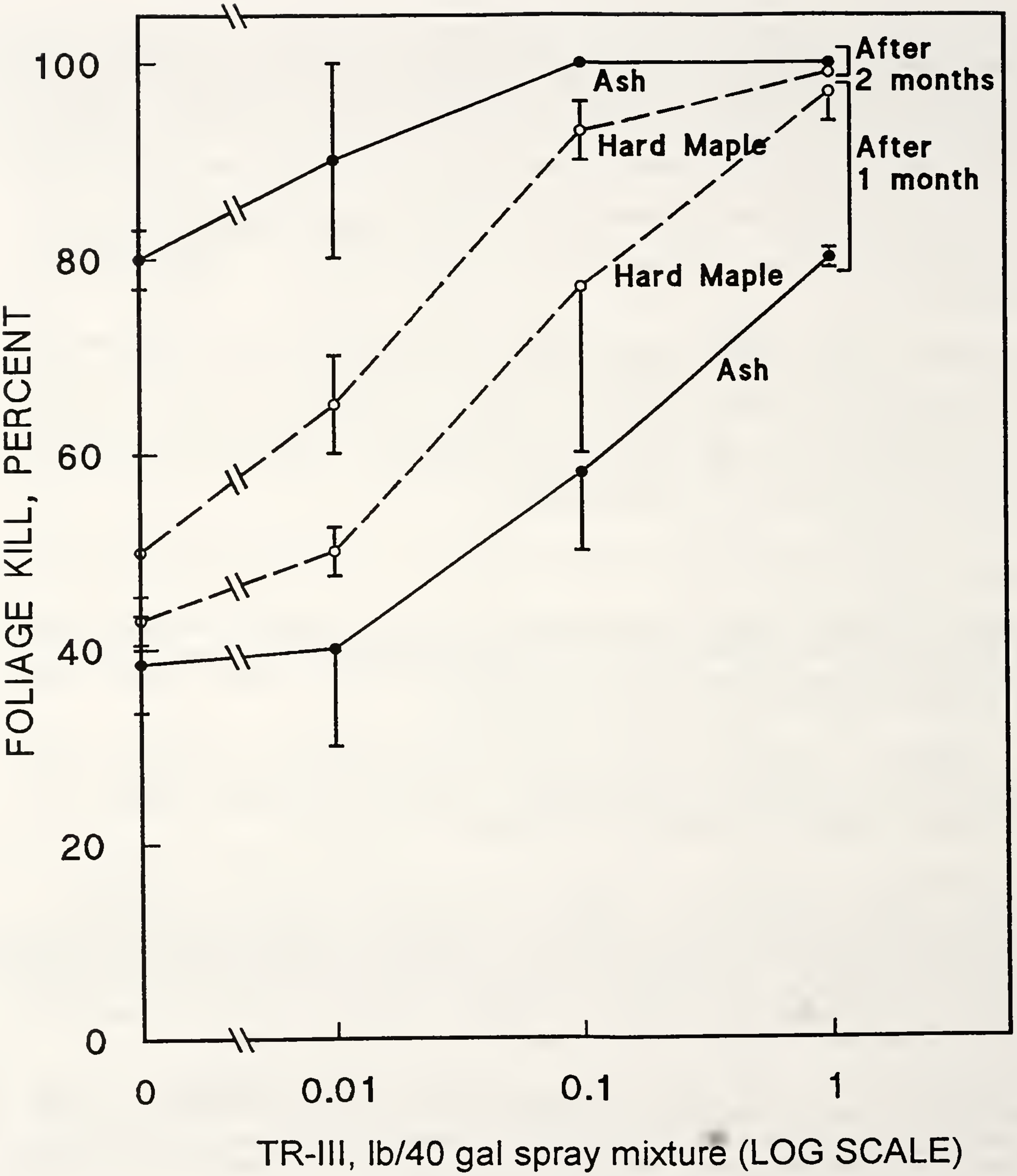
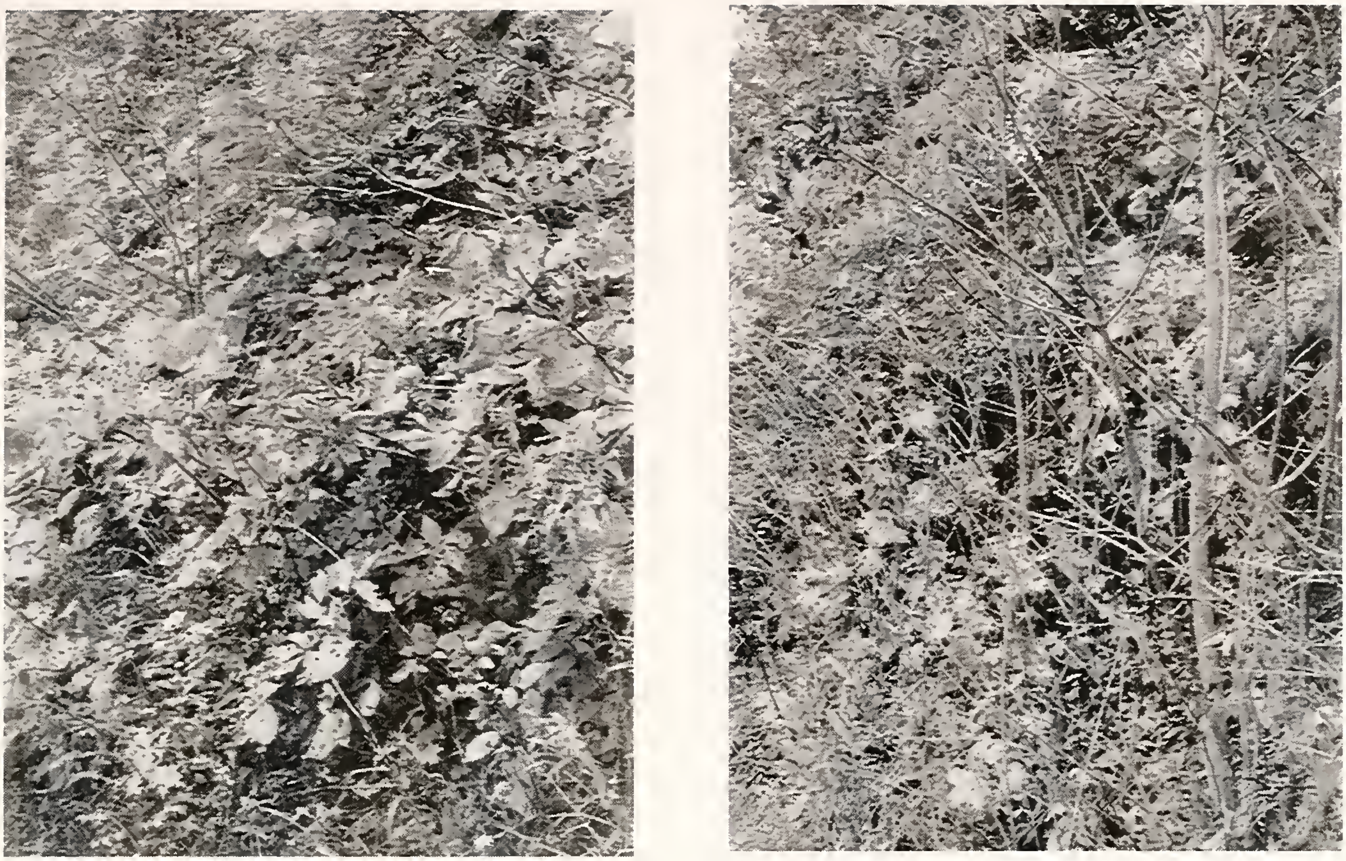
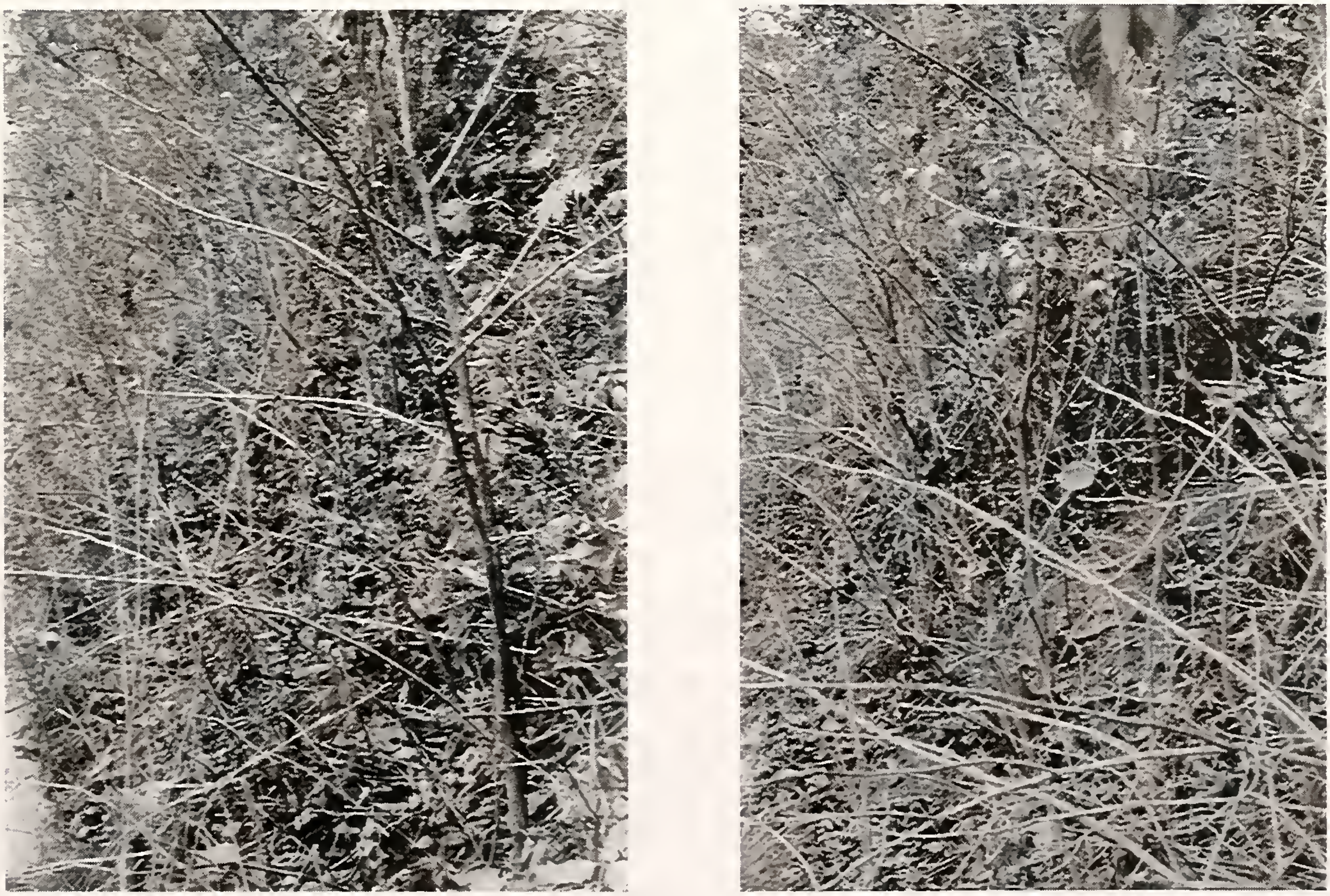


Figure 23. Foliage kill (Defoliation, %) of ash and hard maple determined 1 month and 2 months after spraying with triclopyr in combination with the ammonium nitrate additive and varying rates of the additive TR-III (Cysteine). With the TR-III additive, 100% kill of the triclopyr plus ammonium nitrate-resistant species ash and hard maple was achieved at an application rate of 0.1 lb/A after 2 months.



A. Unsprayed control

B. Triclopyr (2 lb) plus 0.01 lb TR-III (Cysteine) per 40 gal water



C. Triclopyr (2 lb) plus 0.1 lb TR-III (Cysteine) per 40 gal water

D. Triclopyr (2 lb) plus 1 lb TR-III (Cysteine) per 40 gal water

Fig. 24. IN-126 test area. Sprayed August 10, 1993. Photographed October 14, 1993.

what areas will require treatment.

Category IV. Brush (or trees) in safety (Interstate) fences. Brush in safety fences represents the most prevalent brush problem but one where no easy decision will be reached as to what, if any, form of brush removal is required or desired. The present inclination would seem to be to let it go.

6. To develop methods for rapid evaluation of brush control efficacy to facilitate performance payment of contract applications.

The desirability of performance payment of contract applications for spot control of brush and other woody species as a cost-saving factor is clear. Brush is especially amenable to performance evaluation since the dead or dying woody stems or stumps provide a record of what was there at the time of application.

To facilitate possible implementation of performance payment for these activities by the Division of Maintenance, an ongoing activity of the project was to develop a rational basis for an objective method for rapid evaluation of brush control effectiveness. Basically, the only approach that seems to be realistic is to evaluate all brush as to degree of kill between the edge of the pavement and the fence (or in the fence if part of the area to be treated) or whatever treated areas was specified in the contract. A 0.1 mile segment is regarded as sufficient to provide one evaluation. Brush and woody species should be scored as treated only if it shows definite signs of having been sprayed within a reasonable time after spraying. What is a reasonable time will depend on the material (one week might be sufficient for a foliar application of Krenite but a dormant application of another material might require a delay in evaluation until after regrowth occurred in the spring). Brush would be scored as skipped if there was no evidence of having been sprayed or if so little damage had been done that the treatment would be ineffective. the evaluation should include briars, vines, brambles, small trees and any form of wood vegetation but not only species introduced as roadside plantings unless contractor was specifically requested to overspray these areas. Large trees or where trimming was the objective would be evaluated as a separate category. Also, brush in the fence should be regarded as a problem distinct from that in the safety zone. For the most part, counts can be taken from a moving vehicle with occasional stops to verify "skips".

For the triclopyr (Garlon)-additive combinations in this report, the following criteria are recommended. Because the materials tend to work slowly, the payment evaluation should be delayed until at least one month after spraying but should be completed before normal leaf drop occurs in the fall. By this time leaves on treated brush will be brown and easy to recognize even at normal driving speeds. As a convenient rule, if the foliage is more than 50% dead, the treatment may be regarded as having been effective. If the foliage is unharmed or less than 50% dead, the tree was either untreated or treated with insufficient material to be effective. The rating scale should be applied only to treatment combinations that are effective. Resistant species such as juniper and green briar should be excluded from the evaluation. Payment should be limited to that portion of the existing brush (calculated as a percent of total) where greater than 50% of the foliage is dead or dying in the month following treatment.

7. Evaluation of materials for spot control of noxious weeds such as Canadian thistle and for milkweed eradication.

A spectrum of available materials were evaluated during the first two years of the project on the control of Canada thistle and common milkweed, two of the most prevalent weed problems found on Indiana roadsides. In addition to these species, treatments were evaluated as well on whorled milkweed, wild carrot, wild lettuce, ragweed, marestail and other unsightly, tall-growing summertime roadside weeds. New, potentially more effective materials were compared with standard roadside materials, 2,4-D, dicamba (Banvel) and Escort (Telar/Glean) plus picloram (Tordon). The experiments consisted of three experimental series, each comparing 7 to 12 different combinations at three different stages of application (early growth, midflowering and after flowering for thistle and milkweed and at comparable times for other species) and at 3 different locations. These data are included at Appendix II of this report.

For control of milkweed, triclopyr (Garlon), as well as picloram (Tordon K) were found to be effective. However, when the two materials were combined an antagonism was seen. As expected, the standard herbicide mixtures for roadside use such as dicamba (Banvel), 2,4-D or chlorsulfuron (Telar or Glean) were only marginally effective against thistle and milkweed. None of the treatments were effective against whorled milkweed and none of the new materials were more effective against general roadside weeds or Canadian thistle than the standard materials currently in use. Therefore the need for improved herbicide combinations for control of roadside perennial broad leaf weeds and grasses remains.

Indications from laboratory and greenhouse studies are that the TR series of additives may have exceptional promise as new agents in continuing efforts to control problem perennial roadside weeds. The common annual and biennial roadside weeds are being controlled by the current program involving the use of the auxin herbicide, 2,4-D. However, this treatment is unable to effectively eradicate many of the perennials including milkweed, Canadian thistle, bindweed, horsenettle, ground cherry and others. As part of a proposal for a continuing program of roadside maintenance using herbicides and growth regulating molecules will be an evaluation of these additives in combination with both 2,4-D and triclopyr (Garlon) for the control and/or eradication of problem roadside perennial weeds.

8. Implementation trials to evaluate the use of grass growth retardant mixtures for grass seed head control, for use on secondary roads and in roadside chemical trimming operations around signposts and guard rails to reduce mowing costs.

These implementation tests were completed during the first two years of the project and concerned an aspect of a previous project that was new and one where considerable cost savings might have been possible.

A number of trials were completed and evaluated. Generally, such a wide range of weed problems were encountered on secondary roads to preclude the effective use of growth retardant materials even for trimming operations. These problems and possible solutions attempted are summarized in the paragraphs that follow. Also pursued vigorously was an attempt to utilize pre-emergence herbicides to reduce late germinating annual weeds and grasses that contribute more to the unsightly appearance of secondary roads than do the roadside grasses. (Figs. 25-27). These materials, alone or in combination with standard retardant mixtures (Tables 25-27), were only marginally effective (Table 29-33) and the results would not justify the additional costs. As a result, the testing of grass growth retardants for use on secondary roads was discontinued.

Total vegetation management along dual lane highways in Indiana using chemicals and without supplementary mowing was attempted over a nearly 10 year investigative period. During this time, the problem was subdivided into the following subsets:

1) Seed head and vegetative growth control of the dominant roadside grass species - bluegrass and fescue.

2) Seed head and vegetative growth control of minor roadside species - chiefly smooth brome and orchardgrass.

3) Control of broad-leaf perennial and winter annual weeds.

4) Control of late-germinating annual weeds and grasses.

5) Seed head and vegetative growth of late-flowering native species such as paspalum and grease grass.

At the turnout, if any of the above are not controlled to a significant degree, at least supplementary mowing will be required.

Progress during the first years of the project produced economically affordable and effective mixtures of growth retardants that gave full-season control of seed heads and vegetative growth of bluegrass and fescue. They have been used successfully on Interstates where these species dominate and are the subject of published report (Morré and Tautvydas, 1984).

Control of the minor grass species, smooth brome and orchard grass, proved more difficult. However, a tank mix including post and crop oil in addition to the standard mixture of retardant chemicals did give control of these species but at significantly increased cost.

Control of broad-leaf perennial and winter annual weeds was achieved by including 2,4-D in the mixture which proved to be a very cost-effective addition.

Control of late-germinating annual weeds and grasses, however, remains a problem. Using what may be the best pre-emergence materials currently available, the optimum application times for roadside spraying for seed-head control are too early to apply pre-emergence materials for control of annual foxtails, for example. Conversely, the optimum application times for pre-emergence materials for control of annual foxtails comes too late in the season to apply materials for control of seed heads in bluegrass and fescue. Until longer lasting pre-emergence materials can be identified, the emergence of late-germinating foxtails and other weedy grasses preclude cost-effective use of grass growth retardants

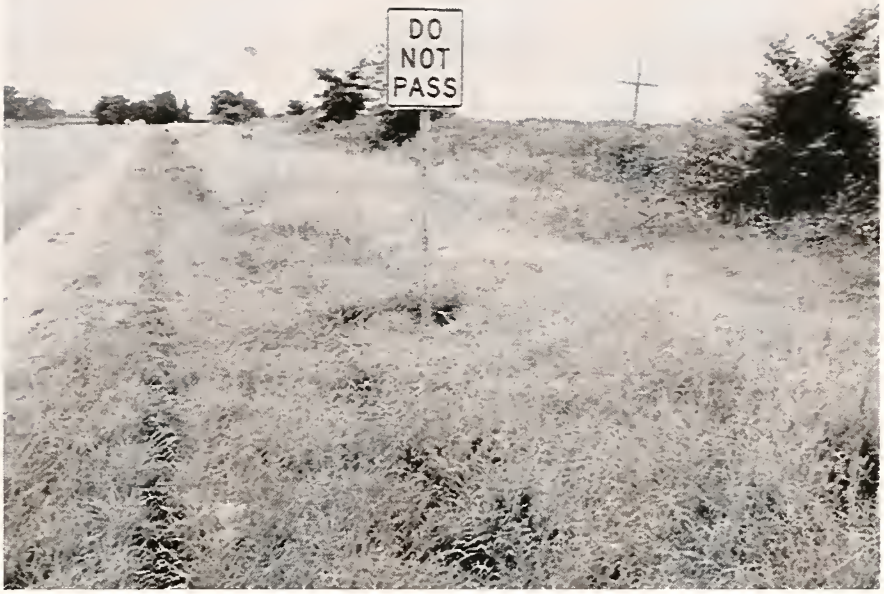


Figure 25. Standard growth retardant mixture (Schedule B, Table 25) used successfully to trim around sign posts to reduce the need for trimming.

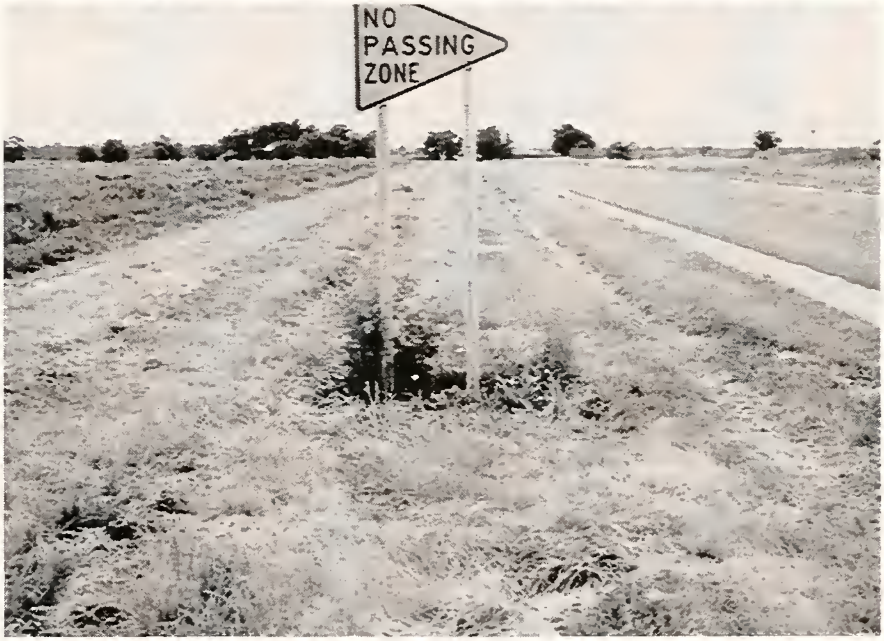


Figure 26. On secondary roads, late emergence of annual weeds and grasses severely compromises the use of chemical retardant mixtures and usually one mechanical mowing cycle is required under the best of conditions when Schedule B or even one of the TR schedules (Tables 26 and 27) is used on secondary roads.

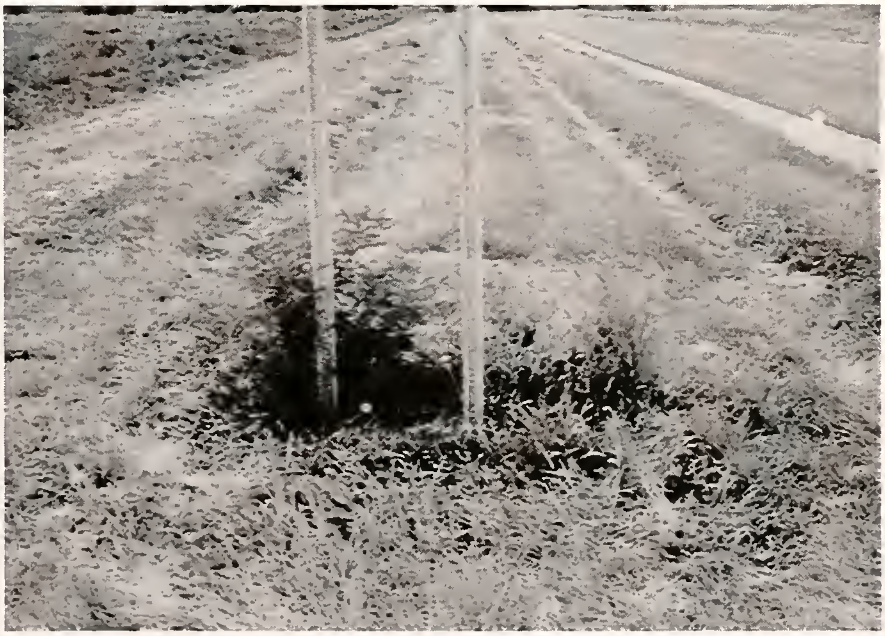


Figure 27. Efforts to control the late emergence of annual weeds and grasses (Tables 28-32) were only partially effective and the results were not sufficiently promising to justify the additional cost of the application.

Table 26. Recommended program of chemical mowing for roadside vegetation management in Indiana. Each of the following schedules has been tested in at least three successive years in at least 3 different locations within the state under actual use conditions with consistent and reproducible results. The 3 schedules normally give equivalent results. Schedule A is the most costly but has the greatest safety margin. Schedule C is the least costly but seed head control in fescue may drop below 90% with some early applications. Schedule B is, at present, considered as the most cost-effective compromise for general highway use.

Materials: Embark (mefluidide) Plant Growth Regulator containing 2 lb active mefluidide per gallon. Telar (chlorsulfuron) concentrate containing 75% active material. 2,4-D amine form concentrate containing 4 lb/gallon acid equivalent (Ester formulations of 2,4-D are not recommended due to possible environmental hazards). X-77 or WK Surfactant concentrate.

Rate:

Schedule A: 1/2 lb/A Embark (as mefluidide) + 0.25% X-77 + 2 lb/A of 2,4-D amine.

Materials are mixed at the rate of approximately 2.3 gallons of Embark plus 1/4 gallon of X-77 plus 1 1/2 gallons of 2,4-D amine in 100 gallons of water. The mixture is applied at the rate of 40 gallons per acre.

Schedule B: 1/4 lb/A Embark (as mefluidide) = 1 pint/A + 1/4 oz/A Telar + 0.25% X-77 + 2 lb/A 2,4-D amine.

Materials are mixed at the rate of approximately 2 1/2 pints of Embark plus 5/8 oz Telar (reduction to 1/2 oz would seem feasible on the basis of 1986 and 1987 test results) plus 1/4 gallon of X-77 plus 1 1/2 gallons of 2,4-D amine in 100 gallons of water. The mixture is applied at the rate of 40 gallons per acre.

Schedule C: 1/8 lb/A Embark (as mefluidide) + 1/2 pint/A + 1/8 oz/A Telar + 0.25% X-77 + 2 lb/A 2,4-D amine.

Materials are mixed at the rate of approximately 1 1/4 pints of Embark plus 5/16 oz of Telar (reduction of 1/4 oz would seem feasible on the basis of 1986 and 1987 test results) plus 1/4 gallon of X-77 plus 1 1/2 gallons of 2,4-D amine in 100 gallons of water. The mixture is applied at the rate of 40 gallons per acre.

Schedule of Application: Recommended for application in the spring only. For schedules A and B, apply as the grass begins to green and grow at the beginning of April until just before the emergence of seedheads from the boot at the end of the first week of May. Application of schedule C should begin approximately 10 days later on or about April 10 with termination the same as schedule B. With schedule A, termination should be about 1 week earlier at the beginning of May. These dates are for the central 1/3 of the state. For the Northern 1/3, one week may be added. For the Southern 1/3, one week may be subtracted.

Table 27. Approximate costs of materials for comparisons for the various recommended and alternate programs of chemical mowing for vegetation management along Indiana roadsides.

Based on: Embark $32.00/lb; Telar $14.50/oz; Oust $8.75/oz; Poast $25.00/lb; Crop Oil $3.00/gallon; Manage $44.00/lb; Garlon-4 $15.00/lb; 2,4-D amine $1.70/lb; X-77 surfactant $10.00/gallon.

	Material cost per acre									
Schedule	Embark	Manage	Telar	Oust	Garlon-4	Poast	Crop Oil	X-77	2,4-D Amine	Total
Recommended Interstate/Dual Lane										
A	$16.00							$1.00	$3.40	$20.40
B	8.00							1.00	3.40	15.40
C	4.00							1.00	3.40	9.90
Alternative Interstate Dual Lane										
D	8.00			$1.20				1.00	3.40	13.50
E	4.00			0.55				1.00	3.40	8.95
F		$11.00	$3.00					1.00	3.40	18.40
G		11.00		1.10				1.00	3.40	16.50
Alternative Single Lane										
SR-1	8.00		3.00		$7.50			1.00		19.50
SR-2	8.00		3.00			$12.50	$3.00	1.00	3.40	30.90*
SR-3		11.00	3.00		7.50			1.00		22.50
SR-4		11.00		1.10	7.50			1.00		20.60

*Costs could drop as low as $21.00 depending on final rates of Poast and 2,4-D.

Table 28. Approximate dates of application for the recommended program of chemical mowing for vegetation management along Indiana roadsides.

	Southern 1/3		Central 1/3		Northern 1/3	
Schedule	Begin	End	Begin	End	Begin	End
A	March 25	April 24	April 1	May 1	April 8	May 8
B	March 25	May 1	April 1	May 8	April 8	May 15
C	April 3	May 1	April 10	May 8	April 17	May 15
D	March 25	May 1	April 1	May 8	April 8	May 15
E	April 3	May 1	April 10	May 8	April 17	May 15
F	April 17	April 24	April 24	May 1	May 1	May 8
G	April 17	April 24	April 24	May 1	May 1	May 8
SR-1	March 25	May 1	April 1	May 8	April 8	May 15
SR-2	March 25	May 1	April 1	May 8	April 8	May 15
SR-3	April 17	April 24	April 24	May 1	May 1	May 8
SR-4	April 17	April 24	April 24	May 1	May 1	May 8

These dates should be regarded as approximate and the application window may vary as much as 7 to 10 days in length depending upon the season and particular weather conditions of any given year.

Table 29. 1990 Implementation trial. Standard mixture of 1/4 lb/A Embark plus 1/4 oz/A Telar with X-77 surfactant (0.25% of total spray mixture). Applied at the rate of 25 gal/A the last week of April 1990. Evaluation was on August 11, 1990. SR-26 westbound from West Lafayette to the Illinois state line.

Smooth brome Seed heads			Fescue Seed heads			Blue grass Seed heads		
Per ft^2	Height	Blade height	Per ft^2	Height	Blade height	Per ft^2	Height	Blade height
1 ± 1	31 ± 1	20 ± 1	1 ± 1	25 ± 3	21 ± 3	0 ± 0	-	18 ± 2

Orchard grass Seed heads			Plants per ft^2		
Per ft^2	Height	Blade height	Yellow foxtail	Giant foxtail	Crabgrass
2 ± 2	23 ± 2	22 ± 2	7 ± 1	6 ± 2	12 ± 4

Table 30. Effect of addition of pre-emergence materials on seed head suppression of smooth brome, fescue and bluegrass. Treatments applied April 18, 1990. Evaluations were on June 4, 1990. IN-126 test area. Additions were to a mixture consisting of Embark 1/4 lb/A, Telar 1/4 oz/A, Garlon-4 1/2 lb/A and Z-77 surfactant 0.25% of final spray volume.

Added pre-emergent	Smooth brome		Fescue		Bluegrass	
Material, lb/A	Per ft^2	Height	Per ft^2	Height	Per ft^2	Height
None	0 ± 1	20 ± 1	1 ± 1	15 ± 1	1 ± 1	8 ± 1
Surflan, 2 lb/A	3 ± 1	21 ± 1	1 ± 1	15 ± 1	1 ± 1	16 ± 2
Balan, 3 lb/A	1 ± 1	20 ± 2	0 ± 1	(16)	2 ± 1	17 ± 2
Control, no treatment	3 ± 1	25 ± 2	8 ± 2	43 ± 4	2 ± 2	16 ± 2

Table 31. Effect of addition of pre-emergence materials to roadside mixtures for seed head suppression and chemical trimming around sign posts on germination of three common species of annual grasses. Applied April 25 and 26, 1990 by the Crawfordsville Subdistrict under the supervision of Don Bickel. Evaluations were on August 15, 1990. Plots 5-10 miles long. SR-25 south of Lafayette, IN.

		Seed stalks/ft^2		
Roadside mixture	Addition	Crabgrass	Yellow foxtail	Giant foxtail
None	None	7 ± 1	10 ± 4	8 ± 2
SR-1	None	7 ± 1	9 ± 2	9 ± 2
	Balan, 3 lb/A	8 ± 1	8 ± 2	6 ± 2
	Surflan, 2 lb/A	0 ± 0	8 ± 2	8 ± 2
SR-2	None	8 ± 2	10 ± 2	8 ± 2
	Balan, 3 lb/A	6 ± 2	8 ± 2	8 ± 2
	Surflan, 2 lb/A	3 ± 3	8 ± 2	8 ± 2

SR-1: 1/4 lb/A Embark (as mefluidide) + 1/4 oz/A Telar + 1/2 lb/A Garlon-4 + 0.25% surfactant + 1/2 lb/A 2,4-D amine

SR-2: 1/4 lb/A Embark (as mefluidide) + 1/4 oz/A Telar + 1/2 lb/A Poast + 1 gal/A crop oil + 2 1/2 lb/A 2,4-D amine

Table 32. **Embark plus Telar in combination with Garlon-4 for control of smooth brome.** Effect of Surflan and Balan pre-emergence additives. Applied April 18, 1990. Initial height of smooth brome 16 in. IN-126 test area. Evaluation on June 4, 1990.

	Amount per acre					
Treatment	Embark	Telar	Garlon-4	Surfactant X-77	Surflan	Balan
Check	-	-	-	-	-	-
A	1/4 lb	1/4 oz	1/2 lb	0.25%	2 qts	-
B	1/4 lb	1/4 oz	1/2 lb	0.25%	-	3 lb
C	1/4 lb	1/4 oz	1/2 lb	0.25%	-	-

	Smooth brome			Fescue			Bluegrass		
	Seed heads		Blade	Seed heads		Blade	Seed heads		Blade
Treatment	per ft^2	Height	Height	per ft^2	Height	Height	per ft^2	Height	Height
Check	3 ± 1	44 ± 4	22 ± 1	9 ± 1	42 ± 4	19 ± 1	2 ± 2	24 ± 4	16 ± 2
A	3 ± 1	39 ± 4	21 ± 2	1 ± 1	19 ± 3	14 ± 2	0 ± 0	-	14 ± 0
B	1 ± 1	38 ± 2	19 ± 2	0 ± 0	-	15 ± 1	1 ± 1	15 ± 3	13 ± 5
C	0 ± 1	34 ± 1	20 ± 1	1 ± 1	21 ± 5	15 ± 3	1 ± 1	11 ± 4	8 ± 2

Table 33. Date of application and efficacy of Balan (3 lb/A) or Surflan (2 qts/A) on control of annual grasses (foxtail and crabgrass). IN-126 test area.

	Amount/acre			
Date of application	Balan	Surflan	Crabgrass/ft^2	Foxtail/ft^2
Check (all dates)	-	-	25 ± 5	30 ± 10
April 19, 1990	3 lb	-	16 ± 8	
		2 qt		9 ± 1
May 2, 1990	3 lb		4 ± 2	
		2 qt	15 ± 5	11 ± 3
May 9, 1990	3 lb		3 ± 3	
		2 qt		7 ± 1
May 14, 1990	3 lb		5 ± 1	
		2 qt		12 ± 2
May 21, 1990	3 lb		1 ± 1	
		2 qt		12 ± 2
May 29, 1990	3 lb		0 ± 0	
		2 qt		12 ± 2
June 15, 1990	3 lb		0 ± 0	
		2 qt		5 ± 1

along secondary roads even as an alternative to mechanical mowing for trimming around signs and guard rails.

Seed head formation and vegetation growth of late-flowering native prairie species such as grease grass is also a problem that very likely will continue to compromise chemical mowing operations even on interstates. With early applications of growth retardants to suppress bluegrass and fescue and in the absence of any mechanical mowing, these species may actually increase to become a dominant grass species. While not objectionable, late summer mowing would then be required to keep the roadsides from becoming unsightly which defeats the purpose of a chemical mowing program.

9. Environmental safety trials

A series of environmental safety trials were continued as a major investigative effort of the research project. One such trial was in place for 10 years under Indiana use conditions and has not been duplicated elsewhere. The results of that trial, which was completed, are included in this report as follows:

a. Abstract

Three primary growth regulators/herbicides, mefluidide, chlorsulfuron and sulfometuron, alone and in combinations with and without surfactant or 2,4-dichlorophenoxyacetic acid (2,4-D), were applied annually up to rates 8 to 10 times cost-effective rates of application to roadside stands of mixed tall fescue (*Festuca arundinacea* Schreb.) and native bluegrass (*Poa pratensis L*). The plots were not mowed. Applications were during the first week of May prior to elongation of culms bearing seed heads. With all of the materials and at all rates of application the grass had recovered fully by the end of the growing season (August). Even in the final year of the trial, all plots still supported strong stands of perennial grasses. The results show that the growth retardant mefluidide alone or in combination with the sulfonylurea herbicides, chlorsulfuron or sulfometuron can be applied to established turf at cost-effective rates on an annual basis without permanent damage to turf or detrimental carryover of materials.

b. Introduction

A combination of a primary growth retardant, mefluidide, a synergistic additive, chlorsulfuron, a detergent to enhance penetration (X-77) and a herbicide, 2,4-D, has been reported to be effective for full-season suppression of seed head formation in bluegrass - tall fescue stands along roadsides at cost-effective rates of application (Morré and Tautvydas 1986). The treatments, if applied prior to seed head emergence, prevent the elongation of culms bearing seed heads and largely eliminate the need for early or mid-season mechanical mowing. However, to achieve the desired reductions in mechanical mowing on a recurring basis, annual applications of the retardant mixtures would be required.

A series of repeated applications of the retardant mixtures and some of their components were initiated in the spring of 1983 after an initial two years of field testing. After ten years both in small plots and under actual roadside use, no permanent loss of turf viability was observed with any of the treatments.

c. Materials and Methods

Growth retardant and herbicide treatments were applied to established roadside turf both to small plots and to large scale test areas under roadside use conditions. Uniform stands of predominantly tall fescue (*Festuca arundinacea* Schreb.) and native perennial bluegrass (*Poa pratensis L.*) in roadside locations were selected for evaluation.

Small Plots

Small plots were in 3 different locations along a 3 mile segment of State Road 126 in Tippecanoe County, Indiana. Plots were triplicated, 1.8 m X 5.4 m. The plots were along a fence line adjacent to a meadow at two locations and a tilled field at another. The plot borders were mowed two to three times each year (spring, mid-season, fall) as a part of normal roadside maintenance, but the plot areas were not mowed at any time during the trial.

Applications were with a hand-held compressed air sprayer, spraying systems 8004 nozzles, 40 psi and 374 l/ha (40 gpa). The initial applications were on May 3-5, 1983. The annual repeat applications all were between May 3 and May 9 (average date of application of May 5). Foliage was dry at the time of application. The average extended blade height of the fescue was 7 (5-10) cm, that of the bluegrass was 5 (3-9) cm. Applications were prior to the elongation of fescue seed head-bearing culms. Plots were rated for grass kill or changes in species composition at the time of application and the following August. Detailed counts were made in August of 1987, 1990 and 1992.

Large-scale Trials

Large-scale trials of the retardant combinations only were applied to a segment of highway I-70 between Indianapolis and the Illinois state line beginning in the spring of 1984. Applications by licensed Department of Highway applicators were made at 234 l/ha (25 gpa) using a commercial (Swinglok) applicator system. During much of the trial, the treated areas were unmowed but were included in a full-width mowing cycle beginning in 1989.

d. Results

After 10 years of annual applications of growth retardant at 8 to 10 times the rates required for suppression of seed head formation, treated areas still supported normal stands of perennial grasses (Table 34). The very high rates of the two sulfonylureas, chlorsulfuron and sulfometuron, and all but the lowest rates of mefluidide inhibited early vegetative growth as well as seed head formation (> 80%) Yet, both fescue and bluegrass survived.

Although not statistically significant, some trends were noted. At cost effective rates (0.16 to 0.28 kg/ha) of mefluidide, bluegrass appeared to increase in density as fescue seed heads were reduced. However at very high concentrations of mefluidide (1.12 and 2.24 kg/ha) this effect was not seen. At high rates of mefluidide, bluegrass appeared to be killed, but then regrew from

Table 34. Tall fescue (*Festuca arundunacea* Schreb.) and native perennial bluegrass (*Poa pratensis* L.) composition of roadside plots after 10 years of annual treatment with growth retardant/herbicides singly and in combination, for 1.8 m x 5.4 m triplicated plots.

Treatment		Culms/m^2 Fescue	Culms/m^2 Bluegrass
None		77 ± 18	4.0 ± 0.2
Schedule A		68 ± 16	5.2 ± 1.2
Schedule B		80 ± 8	4.7 ± 0.7
Mefluidide*	0.14 kg/ha	88 ± 36	6.9 ± 2.1
	0.28 kg/ha	98 ± 46	6.4 ± 1.6
	0.56 kg/ha	79 ± 38	4.8 ± 0.4
	0.84 kg/ha	76 ± 51	4.4 ± 0.4
	1.12 kg/ha	56 ± 5	3.5 ± 0.5
	2.24 kg/ha	58 ± 6	3.0 ± 0.5
Chlorsulfuron*	8.8 g/ha	68 ± 8	4.4 ± 1.2
	17.6 g/ha	68 ± 4	4.8 ± 1.2
	35.2 g/ha	72 ± 8	4.4 ± 0.4
	52.8 g/ha	64 ± 4	4.4 ± 0.4
	70.4 g/ha	49 ± 20	4.0 ± 1.7
	140.8 g/ha	50 ± 6	5.2 ± 0.6
	280.6 g/ha	56 ± 7	6.5 ± 1.8
Sulfometuron*	4.4 g/ha	73 ± 20	5.8 ± 0.8
	8.8 g/ha	72 ± 16	5.0 ± 0.3
	17.6 g/ha	77 ± 25	5.5 ± 0.8
	26.4 g/ha	70 ± 17	5.0 ± 0.25
	35.2 g/ha	60 ± 8	5.2 ± 1.0
	70.4 g/ha	58 ± 8	5.2 ± 0.8
	140.8 g/ha	58 ± 10	5.5 ± 1.1
	218.6 g/ha	48 ± 16	4.4 ± 1.6

Schedule A = Mefluidide 0.56 kg/ha plus 2,4-D amine 2.24 kg/h plus 0.5% X-77 (as percent of the total spray mixture).

Schedule B = Mefluidide 0.28 kg/ha plus chlorsulfuron 17.5 g/ha plus 2,4-D amine 2.24 kg/ha plus 0.5% X-77

Amounts of materials are of active materials. X-77 is as percent of the total spray mixture. Differences were not significant for any of the treatments ($p < 0.05$) as determined by analysis of variance.

Replicates from each location were averaged. Results are means from the three different locations ± standard deviations among locations. All single materials were compared with and without surfactant included in the spray mixture. There were no observable effects of surfactant on grass survival and the values with and without surfactant were pooled for analysis.

*Cost effect rates are represented by the second and third lowest rates for each compound.

newly sprouted rhizomes.

Neither chlorsulfuron nor sulfometuron appeared to exert any detrimental effects on bluegrass. In fact, the ratio of bluegrass to fescue in the plots appeared to approximately double over the 10 year period. In contrast, fescue densities exhibited a significant numerical decrease with increasing concentrations of both sulfonylureas when compared on a logarithmic scale ($r = -0.83$, $p < 0.003$, beginning at 35.2 g/ha for chlorsulfuron and at 17.6 g/ha sulfometuron. In no instance did any of the treatments appear to be toxic. Results obtained in 1988 and 1990 were similar to those summarized in Table 1.

In large scale trials with schedule A, stand composition also did not change appreciably since 1984. In the sprayed areas, both the bluegrass and fescue were shorter in the spring by less than a decimeter after green up (0.9 decimeters for sprayed bluegrass compared to 1.2 decimeters for unsprayed bluegrass and 1.8 decimeters for sprayed fescue compared to 2.4 decimeters for unsprayed fescue), but both bluegrass and fescue were still present and healthy.

e. **Discussion**

A major concern with the annual use of growth retardant mixtures along roadsides was the possibility that the turf would be weakened and deteriorate progressively. The mixture of materials (schedule B) will continue to be evaluated with repeated applications in two of the original locations. However, it seems highly unlikely that problems would ever arise with any of the materials in the mixture as a result of repeated applications if applied as single agents at cost effective rates ($\leq$ 0.56 g/ha for mefluidide and $\leq$ 17.5 g/ha for the sulfonylureas.

Date of application was important both with mefluidide and with the mefluidide-sulfonylurea mixtures. Both were observed to kill fescue and bluegrass applied as either schedule A or schedule B (Table 1) in very early spring. Toxicity correlated with application during a period of extreme cold where growth was slowed following an extended period of warm weather and rapid growth. Our applications were well after such critical periods. The critical periods when mefluidide or mefluidide plus chlorsulfuron toxicity would have been encountered were approximately 4 to 6 weeks earlier in late March or early April. The growth- or weather-related toxicity was observed to the same extent in areas sprayed for the first time as in plots after 7 annual sprays in early to mid April.

Not ruled out by the study are opportunities for long term or accelerated changes in species composition due to reduced competition from the existing fescue or bluegrass plants in the sprayed areas. In one location, there was significant encroachment of quackgrass (*Agropyron repens* L.) from adjacent cropland in one entire replicate of plots sprayed with sulfonylurea alone. However, to what extent the progress of encroachment was accelerated or retarded by the annual application of sulfonylureas could not be evaluated in the present study. This was, however, the only change in species composition observed in any of the plots. Scattered clumps of smooth brome (*Bromus inermis Leyss*) or orchard grass (*Dachtylis glomerata* L.) remained scattered throughout the period of study with no apparent change in relative numbers.

The materials investigated have been studied widely as components of growth retardant mixtures (Freeborg et al., 1985). However except for the four-year trial reported by Dernoeden (1984), most evaluations in the same location have been based on one or more applications within a single season (Diesburg and Christians, 1989; Walker and Welch, 1989). While chlorsulfuron does appear to carry over from one season to the next (Walker and Welch, 1989), this carry over appears to be insufficient to be detrimental to fescue or bluegrass at rates required for seasonal applications (e.g. 17.5 g/ha) in combination with mefluidide.

References

Dernoeden PH (1984) Four-year response of a Kentucky bluegrass-red fescue turf to plant growth retardants. Agron J 76: 807-813

Diesburg KL, Christians NE (1989) Seasonal application of ethephon, flurprimidol, mefluidide, paclobutrazol, and amidochlor as they affect Kentucky bluegrass shoot morphogenesis. Crop Sci 29: 841-847

Freeborg RP, Morré DJ, Daniel WH (1985) Roadside vegetation management. In: F Lemaire (ed.) Proc 5th Intern Turf Res Conf, Avignon, France. 1-5 July 1985. INRA Pub, Paris, pp. 757-766

Morré DJ, Tautvydas K (1986) Mefluidide-chlorsulfuron-2,4-D-surfactant combinations for roadside vegetation management. J Plant Growth Regul 4: 189-201

Walker A, Welch SJ (1989) The relative movement and persistence in soil of chlorsulfuron, metsulfuron-methyl and triasulfuron. Weed Res 29: 375-383

5. Recommendations and Conclusions

The major accomplishments of the project are as follows:

1) Development of an effective and environmentally safe mixture of trichlopyr (Garlon Herbicide) and ammonium nitrate for one application control of deciduous roadside wood vegetation.

2) Discovery and development of a new, environmentally safe and effective TR series of additives that enhance the action of the auxin herbicides (trichlopyr, 2,4-D, picloram) for inclusion in both the brush control mixture and for possible use for the control of milkweed, canada thistle, bindweed, ground cherry and other perennial, herbicide-resistant roadside weeds.

3) A series of 10-year environmental safety trials to monitor possible adverse effects of continuous herbicide applications. None were found at normal use rates and conditions.

4) Implementation trials under actual use conditions of new grass growth retardant mixtures for grass seedhead control for use on secondary roads and in roadside chemical trimming operations. The use of grass growth retardants on secondary roads was compromised by the late emergence of a variety of annual weeds and grasses such that, at best, perhaps only one mowing cycle was saved by the chemical application (not cost effective). Trials to utilize a broad spectrum of pre-emergence herbicides to prevent germination of the unsightly weeds proved to be cost ineffective as well. The use of grass growth retardants on secondary roads was insufficiently effective to justify the cost.

5) Evaluation of existing herbicide materials for roadside control of milkweed and canada thistle. For control of milkweed, trichlopyr (Garlon Herbicide), or picloram (Tordon Herbicide) were found to be more effective than 2,4-D. None of the treatments were effective against whorled milkweed and none of the new materials tested were more effective against general roadside weeds or canada thistle than the standard materials currently in use. Hopefully, the new TR series of additives will provide the needed materials for the control and/or eradication of these and other problem perennial weeds along roadsides.

6) Implementation activities included extensive evaluation of the new brush control mixtures in the Crawfordsville Subdistrict, final evaluation of grass growth retardants for chemical mowing or in trimming operations on secondary roads and development of a strategy to increase the general availability of the TR series of additives or herbicide formulations containing the TR additives through industrial collaborations.

7. Implementation Suggestions

If fully implemented, the findings will result primarily in improved roadside appearance, reduced maintenance costs and increased safety due to improved sight distances.

In the State of Indiana, about 45,000 acres of roadside are mowed in the contract program with an additional 55,000 acres in force account mowing by State crews. Current cost estimates are about $16 to $20 per acre for limited width mowing and up to $30 per acre for full-width mowing.

Work previously implemented resulted in reductions from 5-cycle mowing to 3-cycle mowing due to improved weed control and more effective timing of mowing operations. Taking an average mowing cost of $10 per acre per cycle, cost savings of 100,000 acres x $20/acre = $2,000,000 annually were realized.

At present, the major justification for full-width mowing is for control of brush and problem weeds. Especially on Interstates and divided highways, slightly more than 50% of the mowing costs are in support of full-width mowing. The availability of an improved program of chemical brush and weed control will greatly reduce or even eliminate the need for full-width mowing at every mowing cycle with additional cost savings of $10 to $15 per acre. If applied only to the contract program, additional cost savings of between $500,000 and $750,000 per year in reduced mowing costs are projected when the research is completed and the findings are fully implemented.

The additive mixture for brush control has already been implemented, has been used widely throughout the state and has been effective. As a result much of the brush along Indiana roadsides has already been treated and eradicated based on findings contained in this report.

The next step will be to implement the TR additive series for control of perennial and noxious problem weeds. A source of additive has been identified and the first lot has been shipped for implementation testing in 1995. Several years will be required to optimize application dates, rates and ratios to achieve maximum cost effectiveness.

A patent application has been filed both for the brush mixture and for the TR additive series.

APPENDIX I

Plant Physiol. (1995) 107: 1285–1291

Selective Inhibition of Auxin-Stimulated NADH Oxidase Activity and Elongation Growth of Soybean Hypocotyls by Thiol Reagents

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The NADH oxidase activity of isolated vesicles of soybean (*Glycine max* cv Williams 82) plasma membranes and elongation growth of 1-cm-long hypocotyl segments were stimulated by auxins (indole-3-acetic acid or 2,4-dichlorophenoxyacetic acid [2,4-D]). The auxin-induced stimulations of both NADH oxidase and growth were prevented by the thiol reagents *N*-ethylmaleimide, *p*-chloromercuribenzoate, 5,5′-dithiobis(2-nitrophenylbenzoic acid), dithiothreitol, and reduced glutathione. These same reagents largely were without effect on or stimulated slightly the basal levels of NADH oxidase and growth when assayed in the absence of auxins. In the presence of dithiothreitol or reduced glutathione, both 2,4-D and indole-3-acetic acid either failed to stimulate or inhibited the NADH oxidase activity. The rapidity of the response at a given concentration of thiol reagent and the degree of inhibition of the 2,4-D-induced NADH oxidase activity were dependent on order of reagent addition. If the thiol reagents were added first, auxin stimulations were prevented. If auxins were added first, the inhibitions by the thiol reagents were delayed or higher concentrations of thiol reagents were required to achieve inhibition. The results demonstrate a fundamental difference between the auxin-stimulated and the constitutive NADH oxidase activities of soybean plasma membranes that suggest an involvement of active-site thiols in the auxin-stimulated but not in the constitutive activity.

Morré et al. (1986) observed that the oxidation of NADH by isolated soybean (*Glycine max*) plasma membranes was stimulated by 2,4-D. This stimulation did not require addition of exogenous electron acceptors such as ascorbate radical or hexacyanoferrate III, and electron transfer presumably was either to oxygen or endogenous electron acceptors associated with the plasma membrane (Morré and Brightman, 1991). That this stimulation was distinct from that of the oxygen burst NAD(P)H oxidase of host defense was indicated by the observation that oxidation of NADPH was not stimulated by auxin (Morré and Brightman, 1991).

The auxin-stimulated NADH oxidase activity was purified from soybean plasma membrane by Brightman et al. (1988) as a complex of three proteins with molecular masses of 36, 55, and 72 kD. The NADH oxidase activity and the plasma membrane NADH:hexacyanoferrate III oxidoreductase did not co-purify and appeared to reside on different proteins (Morré and Brightman, 1991).

The present report demonstrates that the auxin-stimulated component of the NADH oxidase is inhibited by thiol reagents. The auxin-induced elongation growth of hypocotyl segments is inhibited specifically by these same thiol reagents. The findings provide evidence for a fundamental difference between the auxin-induced and the constitutive NADH oxidase activities of plasma membrane vesicles from soybean hypocotyls that also is observed for auxin-induced cell elongation. The difference appears to derive from an involvement of thiols at the active site in the auxin-stimulated but not in the constitutive activity.

MATERIALS AND METHODS

Isolation of Plasma Membrane

Soybean seeds (*Glycine max* L. cv Williams 82) were soaked for 4 h in water and grown in the dark (20–22°C) in moist vermiculite. After 4 to 6 d, 2-cm hypocotyl segments, cut just below the cotyledon, were harvested under diminished room light (0.15 μmol photons s^{-1} m^{-2}) and placed in cold water. Segments (40 g) were chopped with razor blades in 40 mL of homogenization medium (0.3 M Suc, 50 mM Tris-Mes [pH 7.5], 10 mM KCl, 1 mM $MgCl_2$, 1 mM PMSF). The homogenates were filtered through one layer of Miracloth (Chicopee Mills, New York, NY) and centrifuged for 10 min at 6,000*g* (HB-rotor, DuPont, Wilmington, DE). The supernatant was recentrifuged at 60,000*g* (SW 28 rotor; Beckman, Fullerton, CA) for 30 min and the pellets were resuspended in 0.25 M Suc with 5 mM potassium phosphate (pH 6.8). Plasma membrane vesicles were prepared using a 16-g aqueous two-phase partitioning system (Sandelius et al., 1987). Resuspended 60,000*g* pellets were mixed with 6.4% (w/w) PEG 3350 (Fisher), 6.4% (w/w) Dextran T500 (Pharmacia), 0.25 M Suc, and 5 mM potassium phosphate (pH 6.8). After mixing the tubes by 40 inversions, the phases were separated by centrifugation at 750*g* for 5 min. The lower phase was repartitioned with a fresh upper phase, and the two upper phases were repartitioned twice with fresh lower phases. The upper phases were diluted approximately 4-fold with buffer and collected by centrifugation at 100,000*g* for 30 min. The membranes were stored frozen at −70°C prior to assay. The yield was 1 to 2 mg of plasma membrane protein.

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Abbreviations: DTNB, 5,5′-dithiobis(2-nitrophenylbenzoic acid); 2-ME, 2-mercaptoethanol; NEM, *N*-ethylmaleimide; PCMB, *p*-chloromercuribenzoate.

NADH Oxidase Activity

The assay for the plasma membrane NADH oxidase was in 50 mM Tris-Mes buffer (pH 7.0) and 150 μM NADH in the presence of 1 mM potassium cyanide, the latter to inhibit any mitochondrial NADH oxidases contaminating the plasma membranes. The assay was started by the addition of 0.1 mg of plasma membrane protein. The reaction was monitored by the decrease in the A_{340} with A_{430} as reference, using an SLM DW-2000 (SLM-Aminco, Urbana, IL) spectrophotometer in the dual-wavelength mode of operation or a Hitachi model U3210. The change of A was recorded as a function of time by a chart recorder. The specific activity of the plasma membrane was calculated using an absorption coefficient of 6.21 mM^{-1} cm^{-1} and expressed as nmol min^{-1} mg^{-1} protein.

Assays were initiated by addition of NADH and measured over 5 min after an equilibration period with NADH of 10 min to achieve an initial steady-state rate. Unless indicated otherwise, after each subsequent addition the assay was continued for 10 min with the steady-state rate between 5 and 10 min being reported. NADH was added first. With 2,4-D and thiol reagents, order of addition has been indicated for individual experiments. Control rates were linear for 50 min or longer. Unless indicated otherwise, results were averages from duplicate determinations from each of three different plasma membrane preparations ± SD values among the three different preparations.

Growth Studies

For growth studies, hypocotyl segments, 1 cm in length, were harvested from the zone of cell elongation under dim laboratory light (1.5 μmol photons s^{-1} m^{-2}) by cutting 5 mm below the cotyledons. The segments were floated on aqueous solutions, 2 mL/10 sections, containing the test substances. Incubations were in darkness at 24°C for 18 h. Growth was estimated from the increase in length of the segments after 3, 6, 12, and 18 h. Lengths were measured to the nearest 0.1 mm.

Protein

Protein content was determined by the bicinchoninic acid procedure (Smith et al., 1985). Standards were prepared with BSA.

RESULTS

Auxin-Induced Component of NADH Oxidase Activity Inhibited by Thiol-Reactive Reagents NEM, PCMB, and DTNB

NADH oxidase activity of plasma membranes isolated from soybean hypocotyls was stimulated by about 20% in the presence of 0.1 μM 2,4-D and by about 70% by 1 μM 2,4-D or IAA (Table I). After 10 min of pretreatment with 0.5 mM NEM, 1 μM PCMB, or 10 nM DTNB, no stimulation of NADH oxidase activity was observed with either 2,4-D or IAA. The concentrations of NEM, PCMB, and DTNB reported in Table I were determined from dose-response curves (e.g. Fig. 1) to be sufficient to inhibit the auxin-stimulated activity. In contrast to the auxin-stimulated component of the NADH oxidase activity, the basal NADH oxidase activity was not inhibited by any of the thiol reagents.

The dose response for NADH oxidase activity in the presence or absence of 1 μM 2,4-D is given in Figure 1A for NEM and Figure 1B for PCMB. The auxin-induced component of the activity was inhibited as concentrations of NEM approached or exceeded 10 μM. With PCMB (Fig. 1B), the auxin-induced component of the NADH oxidase was eliminated at about 1 μM. With both NEM and with PCMB there was a tendency for the compounds to stimulate the NADH oxidase activity in the absence of auxin, especially at high concentrations (Fig. 1) or with prolonged times of preincubation. Under no conditions was the constitutive NADH oxidase activity observed to be inhibited by thiol reagents. Similar results were obtained with DTNB except that inhibitions were observed in the nanomolar range (Table I).

Auxin-Induced Growth Inhibited by Thiol Reactive Reagents

The rate of elongation growth of the soybean hypocotyl segments was approximately doubled by 10 μM 2,4-D. As with the auxin-induced component of NADH oxidase activity, growth induced by 10 μM 2,4-D (0.8 cm) was inhibited preferentially to the constitutive level (0.4 cm) by both NEM (Fig. 2A) and PCMB (Fig. 2B). With NEM (Fig. 2A) but not with PCMB (Fig. 2B), and to a lesser extent with DTNB (Fig. 2C), constitutive growth eventually was inhibited as well, but only as the NEM concentrations exceeded

Table I. *Inhibition by pretreatment with NEM, PCMB, or DTNB of the subsequent auxin (2,4-D or IAA) stimulation of NADH oxidase of plasma membranes of etiolated soybean hypocotyl segments*

The number of determinations is given in parentheses. Values are means ± SD among determinations.

Addition	Post-Addition	NADH Oxidase
		nmol min^{-1} mg^{-1} protein
None	None	1.06 ± 0.05 (5)[a]
	0.1 μM 2,4-D	1.24 ± 0.3 (5)[a]
	1 μM 2,4-D	1.8 ± 0.2 (3)[b]
	1 μM IAA	1.7 ± 0.2 (4)[b]
0.5 mM NEM	None	1.2 ± 0.1 (3)[a]
	0.1 μM 2,4-D	1.24 ± 0.14 (5)[a]
	1 μM 2,4-D	1.20 ± 0.15 (3)[a]
	1 μM IAA	1.0 ± 0.2 (3)[a]
1 μM PCMB	None	1.22 ± 0.20 (8)[a]
	1 μM 2,4-D	1.33 ± 0.15 (8)[a]
	1 μM IAA	1.2 ± 0.15 (3)[a]
10 nM DTNB	None	1.2 ± 0.1 (3)[a]
	1 μM 2,4-D	1.3 ± 0.1 (3)[a]
	1 μM IAA	1.1 ± 0.2 (3)[a]

[a,b] Values not followed by the same letter were statistically different ($P < 0.05$).

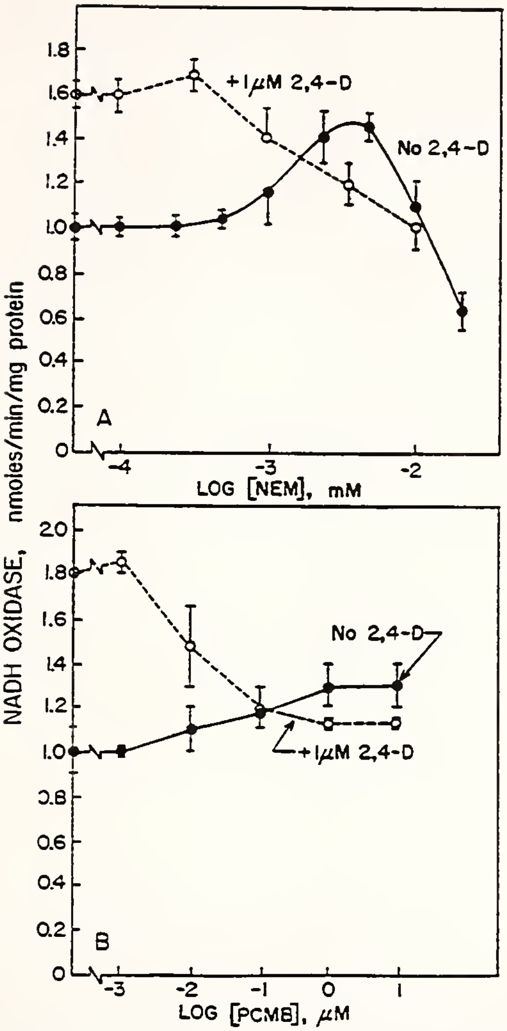

Figure 1. NADH oxidase activity of isolated soybean plasma membranes after 10 min of pretreatment as a function of the logarithm of NEM (A) and PCMB (B) concentration. The thiol reagents preferentially inhibit the 2,4-D-induced stimulation of the oxidase. 2,4-D was added first, followed by NEM or PCMB. Rates are steady-state rates measured 15 min after NEM or PCMB addition. Results are from three experiments with duplicate determinations in each experiment ± SD among the three experiments.

1 mM. Even at 1 mM NEM, where constitutive growth was still unaffected, the auxin-stimulated component of growth was inhibited by about 50%.

In contrast to NEM, PCMB was virtually without effect on the constitutive growth of sections in the absence of auxin (Fig. 2B). However, auxin-induced growth was strongly inhibited by PCMB.

With DTNB (Fig. 2C), inhibition of growth in the absence of auxin was slight and not significant statistically. However, auxin-induced growth was eliminated almost completely. The inhibition of growth over 18 h by 2,4-D was further increased only slightly by first abrading the hypocotyl segments to facilitate cuticular penetration of the DTNB (Fig. 2C, △).

The Auxin-Induced Component of NADH Oxidase Activity Inhibited by GSH and DTT

Differences between the auxin-induced and constitutive NADH oxidase activities became even clearer when responses to GSH and DTT were compared. The NADH oxidase of plasma membrane vesicles in the absence of auxin was largely unaffected by these compounds (Table II). However, the 2,4-D-stimulated increment of NADH oxidase was inhibited completely.

At concentrations of GSH (Fig. 3A) or DTT (Fig. 3B) of 1 and 10 μM, not only was the auxin stimulation of NADH

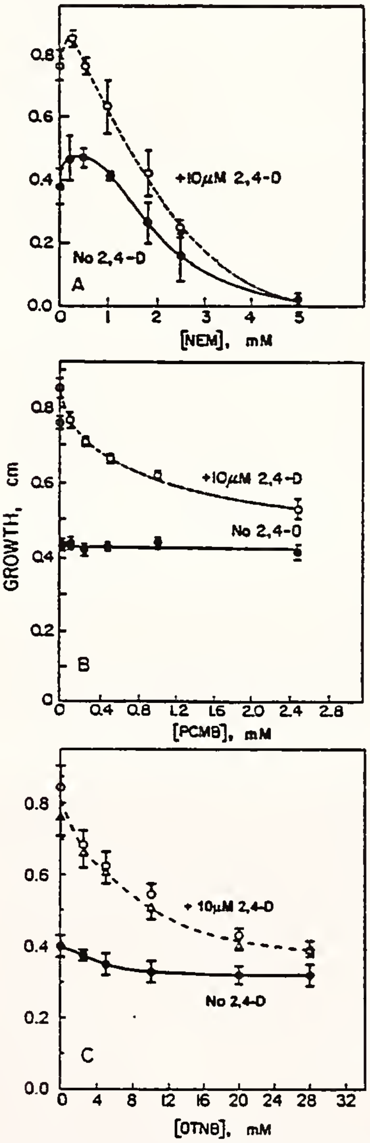

Figure 2. NEM (A), PCMB (B), and DTNB (C) preferentially inhibit auxin-induced (open symbols, dotted lines) elongation of 1-cm segments of etiolated hypocotyls of soybean (cut 0.5 cm below the hook) compared to controls (filled symbols, solid lines). With DTNB (C), comparisons were with abraded hypocotyl segments plus 10 μM 2,4-D (open triangles). Results are means ± SD among experiments of three experiments, each involving measurements of 10 tissue segments incubated for 18 h.

oxidase activity eliminated but the activity in the presence of 1 μM 2,4-D was actually less ($P < 0.01$) than in its absence, i.e. 2,4-D appeared to inhibit rather than to stimulate NADH oxidation in the presence of GSH or DTT. In contrast, the NADH oxidase activity in the absence of 2,4-D was largely unaffected by all concentrations of either GSH or DTT tested.

2-ME did not elicit the same effects on the NADH oxidase as did GSH or DTT. 2-ME was without effect on NADH oxidase activity in the absence of 2,4-D and did not abrogate the 2,4-D or IAA responsiveness of the isolated plasma membrane vesicles at concentrations up to and including 100 μM (Table II).

Auxin-Induced Growth Inhibited by GSH and DTT

Neither GSH nor DTT inhibited growth of excised 1-cm segments of soybean hypocotyls in the absence of 2,4-D (Fig. 4). However, in the presence of 2,4-D, growth was preferentially inhibited by GSH to constitutive levels or below (Fig. 4A). With DTT (Fig. 4B), 2,4-D-stimulated growth also was preferentially inhibited, but not to the same extent as with GSH (Fig. 4A).

2-ME stimulated growth slightly over the range 0.01 to 10 mM and inhibited growth in the presence of 10 μM 2,4-D by about 10% over the same range of concentrations as for GSH (data not shown). GSSG did inhibit auxin-induced growth but at concentrations higher than were required for inhibition of auxin-induced growth by GSH.

Growth Responses Were Steady State

Experiments with each of the growth conditions shown in Figures 2 and 4 were repeated with measurements at 3, 6, 12, and 18 h to verify that growth kinetics were steady state and that steady-state rates were attained within 3 h both in the presence and absence of 2,4-D (Fig. 5).

Table II. *Inhibition by thiol protectants (0.1 mM) of the auxin (1 μM 2,4-D or 1 μM IAA) stimulation of NADH oxidase*

The number of determinations is given in parentheses. Values are means ± SD among determinations.

Addition	Post-Addition	NADH Oxidase
		nmol min⁻¹ mg⁻¹ protein
None	None	1.6 ± 0.15 (7)[a]
	2,4-D	2.3 ± 0.2 (4)[b]
	IAA	2.4 ± 0.2 (4)[b]
GSH	None	1.7 ± 0.3 (5)[a]
	2,4-D	1.4 ± 0.2 (4)[a]
	IAA	1.5 ± 0.1 (3)[a]
DTT	None	1.8 ± 0.3 (5)[a]
	2,4-D	1.4 ± 0.15 (3)[a]
	IAA	1.3 ± 0.1 (3)[a]
2-ME	None	1.6 ± 0.3 (7)[a]
	2,4-D	2.1 ± 0.4 (6)[a]
	IAA	2.0 ± 0.4 (3)[a]

[a,b] Values not followed by the same letter were statistically significant ($P < 0.05$).

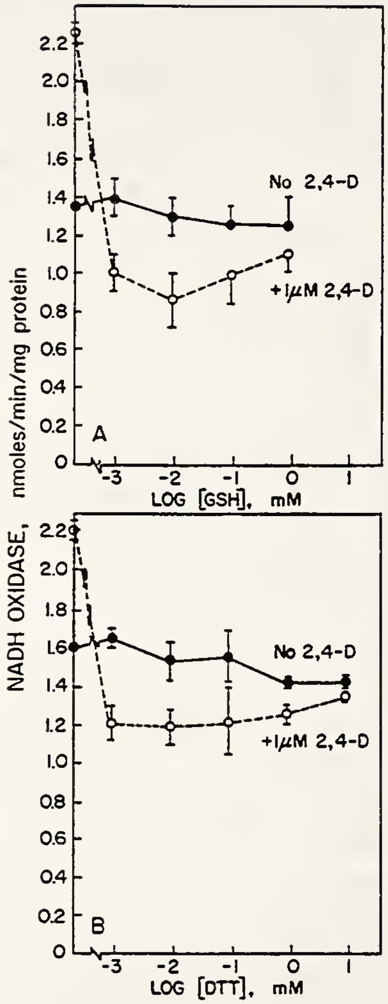

Figure 3. NADH oxidase activity of isolated vesicles of plasma membrane from soybean in response to GSH (A) and DTT (B). The rates shown are steady-state rates following a 5-min pretreatment with the concentrations of GSH and DTT shown with subsequent assay at the same concentrations of GSH and DTT as the pretreatment. Results are means ± SD among experiments of three experiments, with duplicate determinations within each experiment. With membranes pretreated for 5 min with the GSH concentrations shown, the subsequent response to 2,4-D was inhibited.

Preincubation with Auxin Delays Inhibition by Thiol Reagents

Inhibition of NADH oxidase activity by thiol reagents was influenced by the order of reagent addition (Table III). If membranes were first preincubated for 10 min with or without 2,4-D prior to addition of the thiol reagent, 2,4-D stimulation was retained for at least 10 min as if active site thiols were being protected by the 2,4-D. These experiments were conducted together with controls that paralleled the treatments listed in Tables I and II to demonstrate that the thiol reagents did block the stimulation by 2,4-D when the order of addition was reversed. Over 15 min after NEM addition, the apparent protective effects were lost gradually in the presence of 2,4-D (Fig. 6) and were no

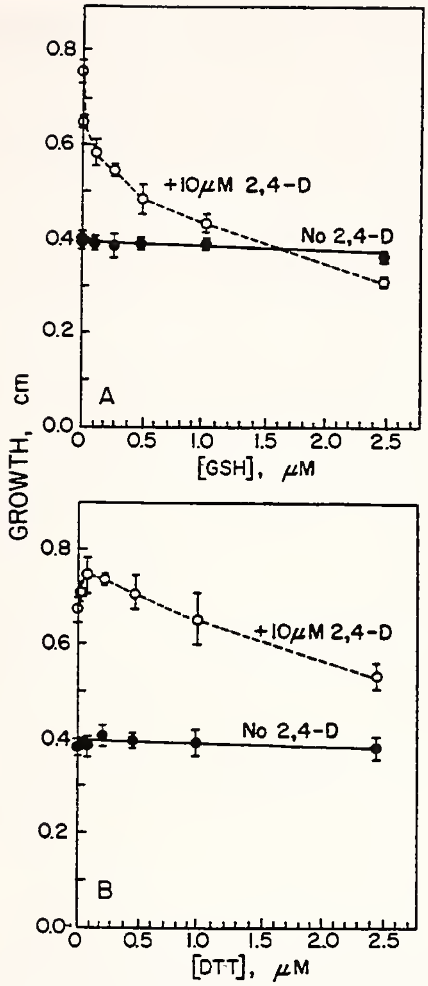

Figure 4. GSH (A) and DTT (B) inhibit auxin (10 μM 2,4-D)-induced but not control (no 2,4-D) elongation of 1-cm segments of etiolated hypocotyls of soybean (cut 0.5 cm below the hook). Results are means ± SD among experiments of three experiments, each involving measurements of 10 tissue segments incubated for 18 h.

longer evident after 10 min of incubation with 10 μM NEM (Fig. 6B). Protection by 2,4-D decreased with increasing concentrations of NEM (Fig. 6B). The NADH oxidase activity in the absence of 2,4-D was largely unaffected by even prolonged treatment with NEM at concentrations of 10 μM or less (Fig. 6A).

DISCUSSION

Studies of the effects of thiol reagents on the auxin-responsive NADH oxidase of soybean plasma membranes have revealed several characteristics of the activity and its regulation by auxin. The results showed that the auxin-responsive component of the oxidase was distinct in its response to thiol reagents from the basal or constitutive activity. The constitutive NADH oxidase activity was resistant to thiol reagents and was not inhibited by NEM, PCMB, DTNB, DTT, or GSH. The 2,4-D-stimulated component of the NADH oxidase, on the other hand, was strongly inhibited by these reagents and reduced or eliminated by concentrations that had little or no effect on the constitutive activity. Thus, it appeared that interference with thiol interchange, prior to or during 2,4-D treatment, either by reaction of free thiol groups of the plasma membrane with NEM, PCMB, or DTNB or by the presence of a redox buffer strongly favoring thiol formation such as DTT or GSH,

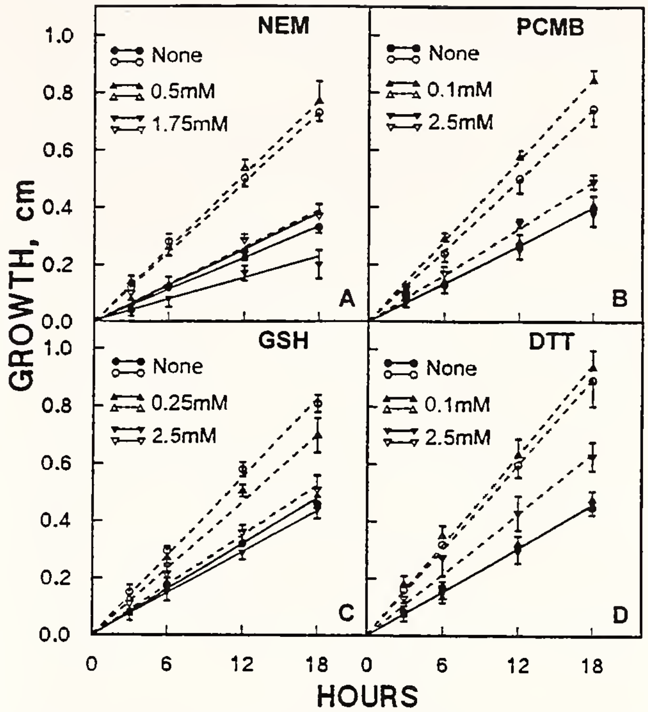

Figure 5. Growth kinetics of 1-cm segments of etiolated hypocotyl of soybean at selected concentrations of NEM (A), PCMB (B), GSH (C), and DTT (D) in the presence (open symbols, dotted lines) or absence (filled symbols, solid lines) of 10 μM 2,4-D. Within the error of the determinations, growth was steady state over the observation period. Results are means ± SD among experiments of three experiments, each involving measurements of 10 tissue segments.

Table III. *Protection by pretreatment with 2,4-D from the action of NEM, PCMB, DTNB, GSH, and DTT in blocking of the auxin (2,4-D or IAA) stimulation of NADH oxidase of plasma membranes of etiolated soybean hypocotyl segments*

2-ME also was tested. Each entry is the average of three determinations. Values are means ± SD among determinations.

Addition	Post-Addition	NADH Oxidase
		nmol min⁻¹ mg⁻¹ protein
None	None	1.15 ± 0.2^{a}
0.1 μM 2,4-D	None	1.35 ± 0.15^{a}
0.1 μM 2,4-D	0.1 mM NEM	1.5 ± 0.2^{a}
1 μM 2,4-D	None	1.95 ± 0.2^{b}
1 μM 2,4-D	0.1 mM NEM	2.0 ± 0.15^{b}
1 μM 2,4-D	1 μM PCMB	1.9 ± 0.23^{b}
1 μM 2,4-D	10 nM DTNB	2.0 ± 0.2^{b}
1 μM 2,4-D	0.1 mM GSH	2.1 ± 0.2^{b}
1 μM 2,4-D	0.1 mM DTT	2.1 ± 0.3^{b}
1 μM 2,4-D	0.1 mM 2-ME	2.2 ± 0.1^{b}

^{a,b} Values not followed by the same letter were statistically significant ($P < 0.05$).

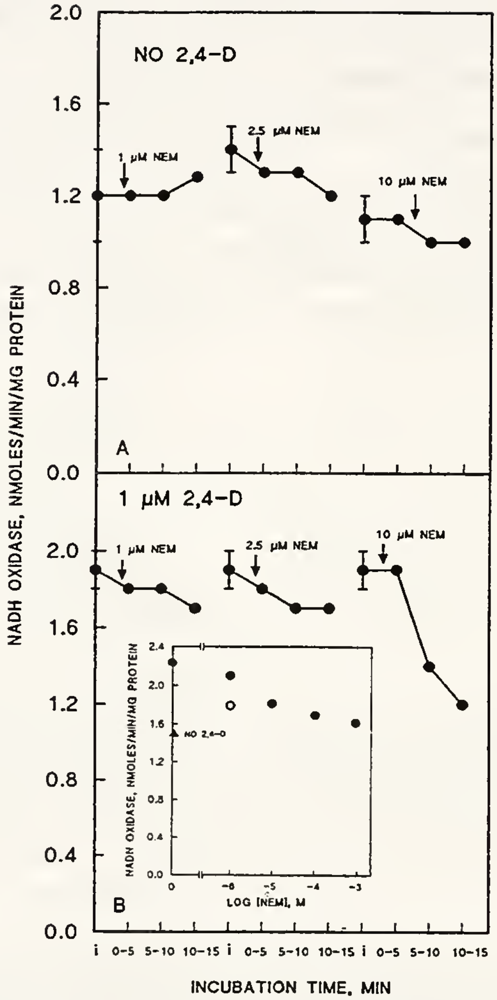

Figure 6. Inhibition by NEM of NADH oxidase activity of soybean plasma membranes as a function of time of incubation and concentration. A, NADH oxidase activity in the absence of 2,4-D. i, Initial steady-state rate after NADH addition. NEM was added as indicated by the arrows and the rate was measured over three successive 5-min intervals. B, As in A except with 1 µM 2,4-D present during the initial incubation with NADH (i). NEM was then added as indicated by the arrows and the rate was again determined over three successive 5-min intervals. Inset, Rate of NADH oxidation in the presence of 1 µM 2,4-D between 5 and 10 min of NEM addition (filled symbols) as a function of NEM concentration. The open symbol is the rate for 1 µM NEM between 10 and 15 min of NEM addition. The 1-µM concentration of NEM had already inhibited 2,4-D-induced NADH oxidase activity after 10 min. The rate without 2,4-D is indicated by the solid triangle.

reduced or prevented the ability of 2,4-D to stimulate the NADH oxidase.

A similar response was seen in the growth of excised stem segments. The 2,4-D-induced growth of soybean hypocotyl segments was much more sensitive to inhibition by DTNB, DTT, PCMB, and GSH than was the basal elongation. With PCMB, for example, basal elongation was poorly inhibited at all PCMB concentrations, whereas auxin-induced elongation was nearly completely inhibited by millimolar concentrations of PCMB. Also inhibitory to 2,4-D-induced elongation of soybean hypocotyls were both GSH and DTT, whereas neither of these compounds elicited a significant response with basal elongation. Only with NEM was the constitutive or basal growth strongly inhibited and only then at very high concentrations. Thus, not only do thiol groups appear to be involved in the response of the plasma membrane NADH oxidase to 2,4-D, but thiol modifications also appear to influence the response of the membranes to 2,4-D. The latter is suggested by the lack of a 2,4-D response after treatment of the membranes with DTT or GSH.

One possible interpretation of the results is that a disulfide bond is required for 2,4-D stimulation of the NADH oxidase activity and that any treatment that prevents disulfide formation interferes with the auxin stimulation. Such a mechanism would be consistent both with the results of Spring et al. (1988) as well as with a mechanism of auxin stimulation of the NADH oxidase based on thiol-disulfide interchange. With the latter, the thiol intermediate of the exchange would be trapped by reaction with NEM or PCMB and the overall activity would be inhibited. At least with NEM there appeared to be an apparent competition between NEM and 2,4-D. This was indicated from the experiments where the membranes were incubated first with 2,4-D and then the sulfhydryl reagents were added. With 2,4-D preincubation, the 2,4-D stimulations were retained for 5 min or longer depending on the sulfhydryl reagent and concentration. However, when the order was reversed and the sulfhydryl reagents were administered first, the 2,4-D stimulations were prevented.

A relationship between auxins and thiols was noted early by Key and Wold (1961) for intact soybean seedlings. They reported that auxins increased the tissue concentration of ascorbic acid, soluble thiols, and protein thiols. Subsequently, Pilet and Dubois (1968) reported a relationship between thiol content and growth rate in vitro. A direct correlation between auxin-induced growth and thiols was demonstrated subsequently for excised hypocotyl segments of soybean using thiol titration methods with silver salts (Morré, 1970). Since these early studies, the role(s) of thiol groups in the plant growth process have received little additional attention, except in the report by Spring et al. (1988), where NEM was suggested to inhibit growth by reacting with thiol groups unmasked by auxin treatment. Reported possibilities for involvements of thiols in the action of mammalian hormones at the plasma membrane include interactions with insulin (Maturo et al., 1983), muscarinic (Aronstam et al., 1978), dopamine (Suen et al., 1980; Sidhu et al., 1986), opiate (Larsen et al., 1981), β-adrenergic (Bottari et al., 1979), vasopressin (Pavo and Farenholz, 1990), leukotirene B4 (Falcone and Aharony, 1990), and peptide (El Battari et al., 1988) receptors.

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LITERATURE CITED

Aronstam RS, Abood LG, Hoss W (1978) Influence of sulfhydryl reagents and heavy metals on the functional state of the muscarinic acetylcholine receptor in rat brain. Mol Pharmacol **14:** 575–586

Bottari S, Vauquelin G, Duriev O, Klutchko C, Strosberg AD (1979) The β-adrenergic receptor of turkey erythrocyte membranes: conformational modification by β-adrenergic agonists. Biochem Biophys Res Commun 86: 1311–1318

Brightman AO, Barr R, Crane FL, Morré DJ (1988) Auxin-stimulated NADH oxidase purified from plasma membranes of soybean. Plant Physiol **86:** 1264–1269

El Battari A, Martin JM, Luis J, Pouzol O, Secchi J, Marvaldi J, Pichon J (1988) Solubilization of the active vasoactive intestinal peptide receptors from human colonic adenocarcinoma cells. J Biol Chem **263:** 17685–17689

Falcone RC, Aharony D (1990) Modulation of ligand binding to leukotirene B_4 receptors on guinea pig lung membranes by sulfhydryl modifying reagents. J Pharmacol Exp Ther **255:** 565–571

Key JL, Wold F (1961) Some effects of 2,4-dichlorophenoxyacetic acid on the oxidation-reduction state of soybean seedlings. J Biol Chem **236:** 549–553

Larsen NE, Mullikin-Kilpatrick D, Blume AJ (1981) Two different modifications of the neuroblastoma X glioma hybrid opiate receptors induced by N-ethylmaleimide. Mol Pharmacol **20:** 255–262

Maturo JM, Hollenberg MD, Aglio LS (1983) Insulin receptor: insulin-modulated interconversion between distinct molecular forms involving disulfide-sulfhydryl exchange. Biochemistry **22:** 2579–2586

Morré DJ (1970) Auxin effects on the aggregation and heat coagulability of cytoplasmic proteins and lipoproteins. Physiol Plant **23:** 38–50

Morré DJ, Brightman AO (1991) NADH oxidase of plasma membranes. J Bioenerg Biomembr **23:** 469–489

Morré DJ, Navas P, Penel C, Castillo FJ (1986) Auxin-stimulated NADH oxidase (semidehydroascorbate reductase) of soybean plasma membrane: role in acidification of cytoplasm? Protoplasma **133:** 195–197

Pavo I, Fahrenholz F (1990) Differential inactivation of vasopressin receptor subtypes in isolated membranes and intact cells by N-ethylmaleimide. FEBS Lett **272:** 205–208

Pilet P-E, Dubois J (1968) Variations du taux en composés sulfhydrilés acidosolubles de tissus cultivés *in vitro*. Physiol Plant **21:** 445–454

Sandelius AS, Barr R, Crane FL, Morré DJ (1987) Redox reactions of tonoplast and plasma membranes isolated from soybean hypocotyls by phase partition. Plant Sci **48:** 1–10

Sidhu A, Kassis S, Kebabian J, Fishman PH (1986) Sulfhydryl group(s) in the ligand binding site of the D-1 dopamine receptor: specific protection by agonist and antagonist. Biochemistry **25:** 6695–6701

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem **150:** 76–85

Spring O, Wolz C, Hager A (1988) Auxin-induced N-ethylmaleimide (NEM) effects on elongation growth of coleoptiles and auxin-induced [^{14}C]NEM labeling of membrane proteins. Physiol Plant **72:** 305–310

Suen ET, Stefanini E, Clement-Cormier YC (1980) Evidence for essential thiol groups and disulfide bonds in agonist and antagonist binding to the dopamine receptor. Biochem Biophys Res Commun **91:** 953–960

APPENDIX II

REPORT SUMMARY

4 January 1990

Work Plan No. 1193. Trichlopyr (Garlon)/ + Chlopyralid (Lontrel)

Three tests were applied: Early (June 24), midseason (July 6) and late (August 5). The early and midseason tests included milkweed, thistle and wild carrot. The late test focused on milkweed only.

With the early test, some control of canada thistle was observed. There appeared as well to be control of common milkweed although why the 1 lb rate of trichlopyr should be less effective than the 0.5 lb rate with or without chlopyralid is difficult to explain. However, similar results were seen for the midseason application where control of canada thistle at the 0.5 lb/A rate of trichlopyr was superior to that with 1 lb/A. These results carried into the 1989 evaluation. Common milkweed control was apparent but percentages of control were again higher with 0.5 lb/A trichlopyr than with 1 lb/A. Whorled milkweed was not controlled by any of the treatments.

The test on August 5 may have been too late for effective milkweed control. None was apparent in the 1989 evaluations. Control of eupatorium (Joe Pye Weed) was enhanced by chlopyralid (0.48 lb/A) added to trichlopyr.

Work Plan No. 1194. Garlon-4 + Tordon K

Three tests were applied: Early (June 6), midseason (July 4) and late (July 28). All three tests included thistle, the midseason included wild carrot and the early and late tests evaluated, in addition, milkweed.

In the early test, there was some initial control of thistle but the rates of both Garlon-4 and Tordon K were too low for either effective thistle or common milkweed control and no control was obvious in the 1989 evaluations. Similar results were seen with the midseason application. The late application was better, averaging about 50% control for canada thistle and somewhat better for common milkweed but the application rates were still too low to provide impressive control of either of these problem roadside weeds. The Dicamba + 2,4-D combination included as a reference mixture was without effect except for easy-to-kill species such as dandelion.

Work Plant No. 01195. Chlopyralid + 2,4-D

Two tests: Applied midseason (June 24) and late (July 22) for control of common milkweed, wild carrot and general roadside weed control. The treatments were ineffective. Escort, included as a comparison treatment, controlled wild carrot as expected.

Future Directions

The different combinations and rates afforded by these trials may not have been optimal for roadside conditions. However, Garlon-4 is, without doubt, a promising material for roadside use and we expect to continue toward making some recommendations either for spot control of thistle or for broadcast applications for control of common milkweed or both. We will probably start at 1 lb/A and add to that Tordon K at 0.25 lb/A with variations around those rates.

EARLY MILKWEED/THISTLE/WILD CARROT TEST. 25% in FLOWER

Milkweed/Thistle/Carrot Test. Work Plan No. 01193. Trichlopyr + Chlopyralid. Applied June 24, 1988.
South River Road (east side) beginning at bridge after CR 575 W.

No	Chemicals	Rate, lb/A	Common Milkweed	Wild Lettuce	Canada Thistle	Wild Carrot	Other Weeds
					Initial		
1	Trichlopyr + Chlopyralid	0.5 + 0.48	10 ± 10	7 ± 7	9 ± 9	18 ± 3	5 ± 5
2	Trichlopyr + Chlopyralid	0.5 + 0.24	16 ± 16	3 ± 3	10 ± 10	30 ± 25	6 ± 5
3	Trichlopyr	0.5	8 ± 8	3 ± 3	8 ± 8	28 ± 28	14 ± 2
4	Trichlopyr + Chlopyralid	1.0 + 0.48	3 ± 1	1 ± 1	6 ± 6	31 ± 31	12 ± 12
5	Trichlopyr + Chlopyralid	1.0 + 0.24	3 ± 1	1 ± 1	7 ± 7	43 ± 43	16 ± 16
6	Trichlopyr	1.0	5 ± 5	3 ± 1	9 ± 9	20 ± 23	11 ± 9
7	Control		5 ± 4	4 ± 2	8 ± 3	8 ± 6	20 ± 17
					Evaluated on July 31, 1989		
1	Trichlopyr + Chlopyralid	0.5 + 0.48	3 ± 2	6 ± 2	0 ± 0	13 ± 7	30 ± 6
2	Trichlopyr + Chlopyralid	0.5 + 0.24	3 ± 2	7 ± 3	1 ± 1	24 ± 12	35 ± 15
3	Trichlopyr	0.5	1 ± 1	12 ± 5	2 ± 4	40 + 24	60 ± 23
4	Trichlopyr + Chlopyralid	1.0 + 0.48	1 ± 1	6 ± 4	1 ± 1	45 ± 21	28 ± 14
5	Trichlopyr + Chlopyralid	1.0 + 0.24	3 ± 2	8 ± 2	1 ± 1	28 ± 20	40 ± 32
6	Trichlopyr	1.0	14 ± 6	7 ± 3	3 ± 2	20 ± 10	35 ± 15
7	Control		3 ± 1	6 ± 4	9 ± 3	22 ± 12	30 ± 10

MILKWEED/THISTLE/CARROT TEST. FULL BLOOM

Milkweed/Thistle/Carrot Test. Work Plan No. 01193. Trichlopyr + Chlopyralid applied July 6, 1988. US 52 North south from Otterbein turnoff.

No	Chemicals	Rate/A	Common Milkweed	Whorled Milkweed	Canadian Thistle	Wild Carrot
1	Trichlopry + Chlopyralid	0.5 + 0.48	100	20	90	12
2	Trichlopyr + Chlopyralid	0.5 + 0.24	72	76	30	12
3	Trichlopyr	0.5	36	62	61	25
4	Trichlopyr + Chlopyralid	1.0 + 0.48	30	230	35	9
5	Trichlopyr + Chlopyralid	1.0 + 0.24	20		160	32
6	Trichlopyr	1.0	62	280	60	30
7	Control	-	50	168	22	17
			Evaluation on July 31, 1989			
1	Trichlopyr + Chlopyralid	0.5 + 0.48	25	18	65	12
2	Trichlopyr + Chlopyralid	0.5 + 0.24	6	52	36	27
3	Trichlopyr	0.5	5	88	0	400
4	Trichlopyr + Chlopyralid	1.0 + 0.48	9	124	60	130
5	Trichlopyr + Chlopyralid	1.0 + 0.24	6		Lost*	3
6	Trichlopyr + Chlopyralid	1.0	6	113	0	40
7	Control	-	10	57	60	17

* Cultivated by adjacent property owner.

LATE MILKWEED TEST, FULL POD

Milkweed Test. Work Plan No. 01193. Trichlopyr + Chlopyralid applied August 5, 1988. US 231 10 miles south of Lafayette, In.

Initial Counts

No	Chemicals	Rate, lb/A	Common Milkweed	Canadian Thistle	Eupatorium	Catnip	Wild Parsnip	Common Mullein	Dogbane	Other	Note
1	Trichlopyr + Chlopyralid	0.5 + 0.48	65	60	22	50	0	1	0	4	1
2	Trichlopyr + Chlopyralid	0.5 + 0.24	23	0	18	17	6	1	0	4	
3	Trichlopyr	0.5	36	0	8	12	0	1	0	2	
4	Trichlopyr + Chlopyralid	1.0 + 0.48	38	0	10	0	0	0	0	10	2
5	Trichlopyr + Chlopyralid	1.0 + 0.24	30	0	0	0	0	2	0	13	
6	Trichlopyr	1.0	26	0	4	5	12	0	40	12	3
7	Control	-	36	0	0	0	12	2	3	22	4

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[1] wild grape/poison ivy

[2] mulberry tree 3 ft high

[3] virgina creeper

[4] poison ivy

Evaluation on September 6, 1989

1.	Trichlopyr + Chlppyralid	0.5 + 0.48	37	96	0	40	13	0	0	3
2.	Trichlopyr + Chlopyralid	0.5 + 0.24	39	0	6	15	8	0	0	2
3.	Trichlopyr	0.5	43	0	5	10		0	0	3
4.	Trichlopyr + Chlopyralid	1.0 + 0.48	48	0	2	2		1	0	8
5.	Trichlopyr + Chlopyralid	1.0 + 0.24	20	0	0	1		1	0	15
6.	Trichlopyr	1.0	18	0	3	6	14	1	50	17
7.	Control	-	47	0	0	0	15	1	4	19

Thistle/Milkweed Test. Work Plan No. 01194. Garlon-4 + Tordon K. Applied June 6, 1988. US 52 north (east side) 0.5 miles north of 231 junction. Early bloom.

			Initial		
No	Chemicals	Rate, lb/A	Canadian Thistle	Common Milkweed	Other Weeds
1	Garlon 4 + Tordon K	0.5 + 0.25	75 ± 38	3 ± 5	5 ± 3
2	Garlon 4 + Tordon K	0.5 + 0.125	97 ± 64	5 ± 6	5 ± 3
3	Garlon 4 + Tordon K	0.5 + 0.063	110 ± 72	3 ± 1	3 ± 2
4	Garlon 4	0.5	88 ± 59	3 ± 3	3 ± 2
5	Garlon 4	1.0	71 ± 39	4 ± 5	4 ± 2
6	Dicamba + 2,4-D	0.5 + 1.0	29 ± 29	6 ± 5	5 ± 1
7	Control		29 ± 12	3 ± 3	5 ± 3
			Evaluation	July 31, 1989	
1	Garlon 4 + Tordon K	0.5 + 0.25	57 ± 18	2 ± 1	14 ± 2
2	Garlon 4 + Tordon K	0.5 + 0.125	75 ± 25	1 ± 1	8 ± 3
3	Garlon 4 + Tordon K	0.5 + 0.063	75 ± 52	1 ± 1	9 ± 3
4	Garlon 4	0.5	76 ± 31	1 ± 1	12 ± 10
5	Garlon 4	1.0	85 ± 39	1 ± 1	11 ± 5
6	Dicamba + 2,4-D	0.5 + 1.0	66 ± 36	2 ± 1	11 ± 6
7	Control		66 ± 28	2 ± 0	14 ± 6

Thistle Test. Work Plan No. 01194. Garlon-4 + Tordon K. Applied July 4, 1988. U.S. 52 North and Klondike Road, Lindberg Road and Klondike Road and Morehouse Road. Thistle was in full bloom.

No	Chemicals	Rate, lb/A	Canadian Thistle	Wild Carrot	Common Milkweed	Other Weeds
			Initial Counts			
1	Garlon-4 + Tordon K	0.5 + 0.25	(90)	8		
2	Garlon-4 + Tordon K	0.5 + 0.125	(35)	47		
3	Garlon-4 + Tordon K	0.5 + 0.063	47	0	0	27
4	Garlon-4	0.5	34	10	0	4
5	Garlon-4	1.0	91	7	0	29
6	Dicamba + 2,4-D	0.5 + 1.0	59	3	3	21
7	Control		234	10	14	61
			Evaluated July 21, 1989			
1	Garlon-4 + Tordon K	0.5 + 0.25	190	64	11	14
2	Garlon-4 + Tordon K	0.5 + 0.125	120	8	1	11
3	Garlon-4 + Tordon K	0.5 + 0.063	25	2	0	13
4	Garlon-4	0.5	80	60	0	13
5	Garlon-4	1.0	125	40	0	21
6	Dicamba + 2,4-D	0.5 + 1.0	130	9	6	15
7	Control		140	6	20	56

Thistle/Milkweed Test. Work Plan No. 01194. Garlon-4 + Tordon K. Applied July 28, 1988. Airport Road, 0.5 miles North of US 26. Plants in full flower at the time of application.

			Canada	Milkweed		Wild	Wild	Other
No	Chemicals	Rate, lb/A	Thistle	Common	Whorled	Parsnip	Lettuce	Weeds
					Initial			
1	Garlon 4 + Tordon K	0.5 + 0.25	70	10	45	62	.5	28
2	Garlon 4 + Tordon K	0.5 + 0.125	60	2	116	12	.1	1
3	Garlon 4 + Tordon K	0.5 + 0.063	140	6	50	6	0	55
4	Garlon 4	0.5	150	6	23	0	3	13
5	Garlon 4	1.0	100	6	0	5	0	41
6	Dicamba + 2,4-D	0.5 + 1.0	122	3	0	3	0	44
7	Control		133	6	0	15	0	25
				Evaluated August 17, 1989				
1	Garlon 4 + Tordon K	0.5 + 0.25	19	2	48	30	4	18
2	Garlon 4 + Tordon K	0.5 + 0.125	42	0	100	10	5	0
3	Garlon 4 + Tordon K	0.5 + 0.063	17	0	62	0		20
4	Garlon 4	0.5	57	1	44	0		50
5	Garlon 4	1.0	18	0	0	0		10
6	Dicamba + 2,4-D	0.5 + 1.0	114	5	0	2		2
7	Control		87	5	0	14	3	30

Milkweed Test. Work Plan No. 01195. Chlopyralid + 2,4-D. Applied June 24, 1988.
South River Road (South Side)

Evaluation August 3, 1989

No.	Chemicals	Rate, lb/A	Common Milkweed	Wild Carrot	Composites	Red Clover	Other Weeds
1	Chlopyralid + 2,4-D	0.48 + 2.5	3 ± 3	8 ± 4	11 ± 3	15 ± 5	79 ± 18
2	Chlopyralid + 2,4-D	0.24 + 1.25	1 ± 1	6 ± 2	17 ± 16	30 ± 19	40 ± 10
3	Chlopyralid + 2,4-D	0.12 + 0.62	1 ± 2	11 ± 8	24 ± 15	23 ± 13	97 ± 48
4	Chlopyralid	0.48	2 ± 2	3 ± 3	22 ± 12	12 ± 8	76 ± 36
5	Chlopyralid	0.24	2 ± 1	6 ± 1	32 ± 12	30 ± 12	117 ± 47
6	Chlopyralid	0.12	1 ± 1	10 ± 9	23 ± 17	24 ± 12	50 ± 10
7	Escort	1/4 oz/A	1 ± 0	1 ± 1	16 ± 3	28 ± 20	60 ± 30
9	Control		1 ± 1	18 ± 9	19 ± 5	22 ± 10	79 ± 18

Work Plan No. 01193. Trichlopyr + Chlopyralid

Three tests: Early, mid and late (June 24/July 6/August 5)

Early and mid on milkweed, thistle and wild carrot; late on milkweed only

Milkweed results will be determined in 1989

Early test, some control of thistle; 0.5 lb rate superior to 1 lb rate/no carrot control

Mid-season test, some control of thistle; 0.5 lb rate superior to 1 lb rate/no carrot control

Results with treatments 3 and 4 are unexplained. No apparent effect on whorled milkweed

Work Plan No. 01194. Garlon-4 + Tordon K

Three tests: Early, mid and late (June 6/July 4/July 28)

Early, mid and late included thistle, mid wild carrot and early and late milkweed

Milkweed results will be determined in 1989

Early test, some control of thistle but rates were too low. Dicamba + 2,4-D was without effect on thistle. Midseason, less effective. Late, also moderately ineffective. All treatments.

Work Plan No. 01195. Chlopyralid + 2,4-D

Two tests midseason and late applied on milkweed and wild carrot. Milkweed results will be determined in 1989. Initial counts were not taken for the June 24 application (mid). Wild carrot, red clover, composites, parsnip, lambs quarters and spurge not controlled.

EARLY MILKWEED/THISTLE/WILD CARROT TEST. 25% in FLOWER

Milkweed/Thistle/Carrot Test. Work Plan No. 01193. Trichlopyr + Chlopyralid. Applied June 24, 1988.
South River Road (east side) beginning at bridge after CR 575 W.

No	Chemicals	Rate, lb/A	Common Milkweed	Wild Lettuce	Canada Thistle	Wild Carrot	Other Weeds
			Initial				
1	Trichlopyr + Chlopyralid	0.5 + 0.48	10 ± 10	7 ± 7	9 ± 9	18 ± 3	5 ± 5
2	Trichlopyr + Chlopyralid	0.5 + 0.24	16 ± 16	3 ± 3	10 ± 10	30 ± 25	6 ± 5
3	Trichlopyr	0.5	8 ± 8	3 ± 3	8 ± 8	28 ± 28	14 ± 2
4	Trichlopyr + Chlopyralid	1.0 + 0.48	3 ± 1	1 ± 1	6 ± 6	31 ± 31	12 ± 12
5	Trichlopyr + Chlopyralid	1.0 + 0.24	3 ± 1	1 ± 1	7 ± 7	43 ± 43	16 ± 16
6	Trichlopyr	1.0	5 ± 5	3 ± 1	9 ± 9	20 ± 23	11 ± 9
7	Control		5 ± 4	4 ± 2	8 ± 3	8 ± 6	20 ± 17
				Evaluated on September 16, 1988			
1	Trichlopyr + Chlopyralid	0.5 + 0.48	10 ± 10		0 ± 0	18 ± 7	29 ± 24
2	Trichlopyr + Chlopyralid	0.5 + 0.24	6 ± 6		1 ± 1	27 ± 27	35 ± 26
3	Trichlopyr	0.5	13 ± 6		3 ± 5	36 + 36	42 ± 13
4	Trichlopyr + Chlopyralid	1.0 + 0.48	7 ± 5		2 ± 2	21 ± 21	37 ± 18
5	Trichlopyr + Chlopyralid	1.0 + 0.24	10 ± 7		2 ± 2	14 ± 14	41 ± 12
6	Trichlopyr	1.0	5 ± 3		3 ± 3	7 ± 7	58 ± 17
7	Control		12 ± 9		8 ± 3	7 ± 6	21 ± 14

MILKWEED/THISTLE/CARROT TEST. FULL BLOOM

Milkweed/Thistle/Carrot Test. Work Plan No. 01193. Trichlopyr + Chlopyralid applied July 6, 1988. US 52 North south from Otterbein turnoff.

No	Chemicals	Rate/A	Common Milkweed	Whorled Milkweed	Canadian Thistle	Wild Carrot*
1	Trichlopry + Chlopyralid	0.5 + 0.48	100	20	90	12
2	Trichlopyr + Chlopyralid	0.5 + 0.24	72	76	30	12
3	Trichlopyr	0.5	36	62	61	25
4	Trichlopyr + Chlopyralid	1.0 + 0.48	30	230	35	9
5	Trichlopyr + Chlopyralid	1.0 + 0.24	20		160	32
6	Trichlopyr	1.0	62	280	60	30
7	Control	-	50	168	22	17
			Evaluation on October 20, 1988			
1	Trichlopyr + Chlopyralid	0.5 + 0.48		no effect	50	12
2	Trichlopyr + Chlopyralid	0.5 + 0.24		tops dead	17	12
3	Trichlopyr	0.5		no effect	0	30
4	Trichlopyr + Chlopyralid	1.0 + 0.48		no control	0	14
5	Trichlopyr + Chlopyralid	1.0 + 0.24		no control	127	3
6	Trichlopyr + Chlopyralid	1.0		no control	60	15
7	Control	-			65	18

* Small carrot also estimated separately. Did not appear to be controlled.

LATE MILKWEED TEST. FULL POD

Milkweed Test. Work Plan No. 01193. Trichlopyr + Chlopyralid applied August 5, 1988. US 231 10 miles south of Lafayette, In.

					Initial Counts						
No	Chemicals	Rate, lb/A	Common Milkweed	Canadian Thistle	Eupatorium	Catnip	Wild Parsnip	Common Mullein	Dogbane	Other	Note
1	Trichlopyr + Chlopyralid	0.5 + 0.48	65	60	22	50	0	1	0	4	1
2	Trichlopyr + Chlopyralid	0.5 + 0.24	23	0	18	17	6	1	0	4	
3	Trichlopyr	0.5	36	0	8	12	0	1	0	2	
4	Trichlopyr + Chlopyralid	1.0 + 0.48	38	0	10	0	0	0	0	10	2
5	Trichlopyr + Chlopyralid	1.0 + 0.24	30	0	0	0	0	2	0	13	
6	Trichlopyr	1.0	26	0	4	5	12	0	40	12	3
7	Control	-	36	0	0	0	12	2	3	22	4

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[1] wild grape/poison ivy

[2] mulberry tree 3 ft high

[3] virgina creeper

[4] poison ivy

Evaluation on October 22, 1988

1.	Trichlopyr + Chlopyralid	0.5 + 0.48		28	dead	40	13	1
2.	Trichlopyr + Chlopyralid	0.5 + 0.24			not dead	15	8	1
3.	Trichlopyr	0.5			o.k.	10		1
4.	Trichlopyr + Chlopyralid	1.0 + 0.48			dead			
5.	Trichlopyr + Chlopyralid	1.0 + 0.24						2
6.	Trichlopyr	1.0			not dead	13	12	
7.	Control	-					12	2

Chlopyralid 0.48 lb/A added to Trichlopyr enhanced control of Eupatorium (Joe Pye Weed), a Composite. Catnip mullein and wild parsnig were not controlled. Milkweed evaluations will be made in 1989.

Thistle/Milkweed Test. Work Plan No. 01194. Garlon-4 + Tordon K. Applied June 6, 1988. US 52 north (east side) 0.5 miles north of 231 junction.

			Initial		
No	Chemicals	Rate, lb/A	Canadian Thistle	Common Milkweed	Other Weeds
1	Garlon 4 + Tordon K	0.5 + 0.25	75 ± 38	3 ± 5	5 ± 3
2	Garlon 4 + Tordon K	0.5 + 0.125	97 ± 64	5 ± 6	5 ± 3
3	Garlon 4 + Tordon K	0.5 + 0.063	110 ± 72	3 ± 1	3 ± 2
4	Garlon 4	0.5	88 ± 59	3 ± 3	3 ± 2
5	Garlon 4	1.0	71 ± 39	4 ± 5	4 ± 2
6	Dicamba + 2,4-D	0.5 + 1.0	29 ± 29	6 ± 5	5 ± 1
7	Control		29 ± 12	3 ± 3	5 ± 3
			Evaluation October 18, 1988		
1	Garlon 4 + Tordon K	0.5 + 0.25	43 ± 18		
2	Garlon 4 + Tordon K	0.5 + 0.125	58 ± 46		
3	Garlon 4 + Tordon K	0.5 + 0.063	63 ± 45		
4	Garlon 4	0.5	65 ± 38		
5	Garlon 4	1.0	65 ± 30		
6	Dicamba + 2,4-D	0.5 + 1.0	41 ± 25		
7	Control		40 ± 23		

Milkweed evaluations will be made in June 1989.

Thistle Test -- Full Bloom

Thistle Test. Work Plan No. 01194. Garlon-4 + Tordon K. Applied July 4, 1988. U.S. 52 North and Klondike Road, Lindberg Road and Klondike Road and Morehouse Road. Thistle was in full bloom.

			Initial Counts			
No	Chemicals	Rate, lb/A	Canadian Thistle	Wild Carrot	Common Milkweed	Other Weeds
1	Garlon-4 + Tordon K	0.5 + 0.25	(90)	8		
2	Garlon-4 + Tordon K	0.5 + 0.125	(35)	47		
3	Garlon-4 + Tordon K	0.5 + 0.063	47	0	0	27
4	Garlon-4	0.5	34	10	0	4
5	Garlon-4	1.0	91	7	0	29
6	Dicamba + 2,4-D	0.5 + 1.0	59	3	3	21
7	Control		234	10	14	61
			Evaluated October 18, 1988			
1	Garlon-4 + Tordon K	0.5 + 0.25	79	6		
2	Garlon-4 + Tordon K	0.5 + 0.125	47	40		1
3	Garlon-4 + Tordon K	0.5 + 0.063	30	1		2
4	Garlon-4	0.5	58	24		0
5	Garlon-4	1.0	127	23		2
6	Dicamba + 2,4-D	0.5 + 1.0	86	0		5
7	Control		185	4		45

Thistle/Milkweed Test. Work Plan No. 01194. Garlon-4 + Tordon K. Applied July 28, 1988. Airport Road, 0.5 miles North of US 26. Plants in full flower at the time of application.

			Initial					
No	Chemicals	Rate, lb/A	Canada Thistle	Milkweed Common	Milkweed Whorled	Wild Parsnip	Wild Lettuce	Other Weeds
1	Garlon 4 + Tordon K	0.5 + 0.25	70	10	45	62	15	28
2	Garlon 4 + Tordon K	0.5 + 0.125	60	2	116	12	1	1
3	Garlon 4 + Tordon K	0.5 + 0.063	140	6	50	6	0	55
4	Garlon 4	0.5	150	6	23	0	3	13
5	Garlon 4	1.0	100	6	0	5	0	41
6	Dicamba + 2,4-D	0.5 + 1.0	122	3	0	3	0	44
7	Control		133	6	0	15	0	25
				Evaluated October 18, 1988				
1	Garlon 4 + Tordon K	0.5 + 0.25	51			36	3	13
2	Garlon 4 + Tordon K	0.5 + 0.125	48			7	5	0
3	Garlon 4 + Tordon K	0.5 + 0.063	89			3	5	36
4	Garlon 4	0.5	135			0		73
5	Garlon 4	1.0	83			0		7
6	Dicamba + 2,4-D	0.5 + 1.0	127			1		1
7	Control		150			13	6	35

Milkweed Test. Work Plan No. 01195. Chlopyralid + 2,4-D. Applied June 24, 1988.
South River Road (South Side)

Evaluation August 19, 1988

No.	Chemicals	Rate, lb/A	Common Milkweed	Wild Carrot	Composites	Red Clover	Other Weeds
1	Chlopyralid + 2,4-D	0.48 + 2.5	2 ± 2	8 ± 6	8 ± 4	16 ± 6	78 ± 62
2	Chlopyralid + 2,4-D	0.24 + 1.25	1 ± 1	15 ± 11	15 ± 11	25 ± 2	41 ± 12
3	Chlopyralid + 2,4-D	0.12 + 0.62	2 ± 2	36 ± 32	23 ± 3	20 ± 4	100 ± 88
4	Chlopyralid	0.48	4 ± 3	9 ± 6	28 ± 17	13 ± 6	62 ± 47
5	Chlopyralid	0.24	2 ± 1	16 ± 6	31 ± 20	47 ± 10	100 ± 33
6	Chlopyralid	0.12	3 ± 3	11 ± 9	26 ± 14	33 ± 25	55 ± 19
7	Escort	1/4 oz/A	3 ± 3	11 ± 7	4 ± 2	28 ± 8	29 ± 27
9	Control		1 ± 1	16 ± 15	21 ± 4	21 ± 5	80 ± 16

Milkweed Test. Work Plan No. 01195. Chlopyralid + 2,4-D. Applied 22 July 1988.
US 52 North, north of 400 W.

No.	Chemicals	Rate, lb/A	Initial counts
1	Chlopyralid + 2,4-D	0.48 + 2.5	wild carrot 75; wild lettuce 25; ragweed 5, mare's tail 15; white top 4; common spurge 30; milkweed 15, willow 3, bindweed 12, small carrot 15/ft^2
2	Chlopyralid + 2,4-D	0.24 + 1.25	wild carrot 42; wild parsnip 8; white top 30; mare's tail 20; poison ivy; common spure 22; multiflora rose 1; willow 6; bindweed 6 ; black milkweed 6; medic 20; dewberrier small carrot 12/ft^2
3	Chlopyralid + 2,4-D	0.12 + 0.62	wild carrot 34; milkweed 8; wild lettuce 7; white top 25; mares tail 22, wild parsnip 10; ragweed 3; common spure 25, poison ivy, lamb's quarter 4; smartweed 1, other weeds 8, 4/ft^2 small carrot
4	Chlopyralid	0.48	wild carrot 23, milkweed 7, wild parsnip 16; dogbane 1; common spurge 35; white top 15; wild lettuce 18; sweet clover 6; buckhorn plantain 9; sumac; poison ivy, willow 6; small carrot 3 /ft^2
5	Chlopyralid	0.24	wild carrot 69; wild parsnip 3; wild lettuce 15; ragweed 6; common milk weed 6: whorled milkweed 30; common spurge 15; white top 16; other weeds 11; small carrot 6/ft^2
6	Chlopyralid	0.12	wild carrot 5; wild lettuce 28; white top 4; wild parsnip 22; poison ivy; multiflora rose: wild grape; ground cherry 2; milkweed 7: common spurge 10; mare's tail 9, other weeds 4; small carrot 2/ft^2
7	Escort	1/4 oz/A	wild carrot 12; white top 15; parsnip 2; mare's tail 21; wild lettuce 26; common spurge 40; common milkweed 2; whorled milkweed 2; dogbane 25; vetch; other weeds 4
8	Control		wild carrot 3; thistle 8; mare's tail 24; wild lettuce 16; white top 15; ragweed 6; spurge 15; wild parsnip 60; buckhorn plantain 6; bindweed; other weeds 12; small carrot 12/ft^2

Evaluation on October 21, 1988

1	Chlopyralid + 2,4-D	0.48 + 2.5	No control of carrot or spurge; milkweed o.k.
2	Chlopyralid + 2,4-D	0.24 + 1.25	As above, some milkweed curled
3	Chlopyralid + 2,4-D	0.12 + 0.62	As above; no control of parsnip, lambs quarter or small carrot

4	Chlopyralid	0.48	No control of carrot or spurge; no control of parsnip, lambsquarter or small carrot
5	Chlopyralid	0.24	As above; no control of sweet clover
6	Escort	1/4 oz/A	Control of carrot; 50% control of vetch
7	Control		wild carrot 1; thistle 6; white top 22; wild parsnip 14; buckhorn plantain 22; dandelion 2; small carrot ca. 12/ft^2

No Control in 1989 evaluations.

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